Guidance for the Preparation of an Interaction Profile

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Public Health Service
Agency for Toxic Substances and Disease Registry
Division of Toxicology

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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found. The Food Quality Protection Act (FQPA) of 1996 requires that factors to be considered in establishing, modifying, or revoking tolerances for pesticide chemical residues shall include the available information concerning the cumulative effects of substances that have a common mechanism of toxicity, and combined exposure levels to the substance and other related substances. The FQPA requires that the Administrator of the Environmental Protection Agency (EPA) consult with the Secretary of the Department of Health and Human Services (which includes ATSDR) in implementing some of the provisions of the act.

To carry out these legislative mandates, ATSDR's Division of Toxicology (DT) has developed and coordinated a research program for chemical mixtures that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development. These efforts are interrelated. For example, the trend testing suggests mixtures of concern for further research, the mixtures toxicological testing contributes to the design and calibration of the models and validation of the methodology, and the modeling and methodology efforts suggest further testing to resolve issues and enhance understanding.

In this manner, ATSDR scientists, in collaboration with mixtures risk assessors and laboratory scientists, have been evolving an approach to the assessment of the joint toxic action of chemical mixtures over a number of years. The approach includes development of Interaction Profile documents to evaluate the joint toxic action of chemicals in simple mixtures that have been identified as being of special concern to ATSDR.

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Disease Registry
BINWOE	Binary weight-of-evidence
BMD	benchmark dose
BTEXs	benzene, toluene, ethylbenzene and xylenes
CDD	chlorinated dibenzo-*p*-dioxin
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DNA	deoxyribonucleic acid
DT	Division of Toxicology
EPA	Environmental Protection Agency
FQPA	Food Quality Protection Act
HI	hazard index
IARC	International Agency for Research on Cancer
ISS	Integral Search System
kg	kilogram
LOAEL	lowest-observed-adverse-effect level
MFO	mixed function oxidase
mg	milligram
MRL	Minimal Risk Level
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
PAH	polycyclic aromatic hydrocarbon
PBPK/PD	physiologically based pharmacokinetic/pharmacodynamic
PCB	polychlorinated biphenyl
ppm	parts per million
QA	quality assurance
QC	quality control
RfC	Reference Concentration
RfD	Reference Dose
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	Toxic Equivalency Factor
TLV	threshold limit value
TTD	target-organ toxicity dose
U.S.	United States
WOE	weight-of-evidence

>	greater than
$	greater than or equal to
=	equal to
<	less than
#	less than or equal to

GENERAL INSTRUCTIONS

These guidelines are designed to assist contractors with preparing interaction profiles for ATSDR.

Purpose and Scope of the Interaction Profile

The purpose of an interaction profile is to evaluate data on the toxicology of a priority mixture and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture minimum risk level (MRL), and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic models (PBPK/PD) for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

The profile provides environmental health scientists with ATSDR DT's recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. A concern in terms of potential impact on public health is whether additivity and toxicological interactions may increase the health hazard above what would be expected from an assessment of each component of the mixture singly. The recommended approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios and the methodology described in the *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures* (ATSDR 2001) to produce site-specific assessments for mixtures. Other users of interaction profiles may be health professionals who need succinct interpretations of the interactions data in order to respond to telephone inquiries from the public.

The interaction profiles are intended to deal with simple mixtures, defined as mixtures containing a relatively small number of chemicals, no more than ten. Interaction profiles provide interpretations of the whole mixture and joint toxic action data. Interpretations are useful for health professionals and

environmental health scientists who may not have the resources to gather and consider all of the relevant data themselves. Interpreting data often requires judgment and implicit assumptions that are more a matter of policy than objective science. Specifically, the profiles incorporate ATSDR DT's evaluations of the validity of particular studies and the inferences that can be made from them.

Introduction to Mixtures, Additivity, and Interactions

The term chemical mixture is used to denote the concept of exposure to two or more chemicals. Chemical mixtures include manufactured mixtures (such as pesticide formulations, gasoline), generated mixtures (byproducts of smelting, drinking water disinfection, fuel combustion), and coincidental mixtures (chemicals deposited separately at a waste site, but having the potential to reach the same "receptor population" by their presence in or migration into the same medium (commonly groundwater) and pathway, or through a combination of media or pathways. These categories of mixtures describe how the mixture originated. (A receptor population is a population that is exposed or potentially exposed through identified exposure routes to contaminants at an exposure point [ATSDR 1992]).

Additional mixture definitions used in assessing the consequences to human health of joint toxic action of chemical mixtures are provided in Table 1.

Toxicological interactions can either increase or decrease the apparent toxicity of a mixture relative to that expected on the basis of dose-response relationships for the components of the mixture. Table 2 provides definitions of terms used in describing the results of joint toxic action studies. These definitions are used in the *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures* (ATSDR 2001) and will be used in this document as well; other definitions exist. Interactions are defined as deviations from the results expected on the basis of additivity.

Table 1. Definitions of Chemical Mixture Terms*

Mixture	Any combination of two or more chemicals, regardless of source and spatial or temporal proximity, that may jointly contribute to actual or potential effects in a receptor population.
Simple Mixture	A combination of a relatively small number of chemicals (no more than 10) that have been identified and quantified (e.g., the components of concern for a receptor population near a hazardous waste site may constitute a simple mixture).
Complex Mixture	A combination of so many chemicals that the composition of the mixture is not fully characterized, either qualitatively or quantitatively, and may be variable (e.g., cigarette smoke, diesel exhaust, gasoline).
Similar Mixtures	Mixtures having the same chemicals but in slightly different proportions or having most but not all chemicals in common and in highly similar proportions. Similar mixtures are expected to have similar fate, transport, and health effects (e.g., the jet fuel JP-5 from different sources).
Chemical Class	A group of chemicals that are similar in chemical structure and biological activity, and which frequently occur together in the environment, usually because they are generated by the same process, such as manufacturing or combustion (e.g., polychlorinated biphenyls [PCBs], chlorinated dibenzo-*p*-dioxins [CDDs]).
Components	The chemicals that make up a mixture.
Components of Concern	The chemicals in a mixture that are likely contributors to health hazard either because their individual exposure levels exceed health guidelines, or because joint toxic action with other components, including additivity or interactions, may pose a health hazard.
Index Chemical	The chemical selected as the basis for standardization of toxicity of components in a chemical class (e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD] for the assessment of dioxin-like compounds; benzo[a]pyrene for the assessment of carcinogenic polycyclic aromatic hydrocarbons [PAHs]).
Indicator Chemical(s)	A chemical (or chemicals) selected to represent the toxicity of a mixture because it is characteristic, potent, and has adequate dose-response data (e.g., benzene has been suggested as an indicator chemical for gasoline).

*Modified from EPA 1986, 1990, 1999; Fay and Feron 1996; Hertzberg et al. 1999.

Table 2. Interactions Terminology[a,b]

Interaction	When the effect of a mixture is different from additivity based on the dose-response relationships of the individual components.
Additivity	When the effect of the mixture can be estimated from the sum of the exposure levels (weighted for potency) or the effects of the individual components.
No apparent influence	When a component which is not toxic to a particular organ system does not influence the toxicity of a second component on that organ system.
Synergism	When the effect of the mixture is greater than that estimated for additivity on the basis of the toxicities of the components.
Potentiation	When a component that does not have a toxic effect on an organ system increases the effect of a second chemical on that organ system.
Antagonism	When the effect of the mixture is less than that estimated for additivity on the basis of the toxicities of the components.
Inhibition	When a component that does not have a toxic effect on a certain organ system decreases the apparent effect of a second chemical on that organ system.
Masking	When the components produce opposite or functionally competing effects on the same organ system, and diminish the effects of each other, or one overrides the effect of the other.

[a]Where effect is incidence or measured response, and additivity commonly is dose or response additivity.
[b]Based on definitions in EPA (1990, 1999), Mumtaz and Hertzberg (1993), and Hertzberg et al. (1999).

Additivity. A default assumption commonly used in mixtures risk assessment is that the chemicals in the mixture act additively. Additivity is commonly defined as dose addition or response addition. Definitions of dose and response addition are provided below, and more detailed information, including equations, is provided in Appendix A of ATSDR (2001).

Dose Addition, also known as concentration addition, simple similar action, and similar joint action, assumes that the components of a mixture behave as concentrations or dilutions of one another, differing only in their potencies (Bliss 1939; Finney 1971). The dose-response curves are parallel (i.e., the regression lines of probits on log doses are parallel), and tolerance (or susceptibility) to the components is completely positively correlated (the organisms most susceptible to chemical A also will be most susceptible to chemical B). The response to the mixture can be predicted by summing the doses of the components after adjusting for the differences in potencies. Dose addition is considered most appropriate

for mixtures with components that affect the same endpoint by the same mode of action (EPA 1986, 1990, 1999). It has been suggested that the requirement for parallel dose-response curves and complete correlation of tolerances may be too stringent (e.g., Plackett and Hewlett 1952; Svendsgaard and Hertzberg 1994), and that in the low-dose region in which the response is linear, dose additivity may hold for independently acting chemicals as well (Svendsgaard and Hertzberg 1994). Dose addition is the underlying assumption of the hazard index method and the toxic equivalency factor (TEF) approach.

Response Addition, also known as simple independent action and independent joint action (Bliss 1939), assumes that the chemicals act independently and by different modes of action. Tolerance (or susceptibility) to one component may or may not be correlated with tolerance to another. The organisms most susceptible to chemical A may also be most susceptible to chemical B (complete positive correlation) or may be least susceptible to chemical B (complete negative correlation), or the susceptibilities to the two chemicals may be statistically independent. The response to the mixture can be predicted from the responses to the components and the correlation of tolerances. Response addition is the underlying assumption of an approach to cancer risk assessment for mixtures and American Conference of Governmental Industrial Hygienists' (ACGIH) approach to assessing the hazard of occupational exposure to agents that act independently.

Interactions. Interactions, that is joint actions that differ from additivity, are categorized as less than additive (antagonism, inhibition, masking) or greater than additive (synergism, potentiation). The non-interaction category is additivity and no apparent influence. The direction of the interaction with respect to additivity is an essential feature of the interaction for mixtures health or risk assessment. The mechanistic bases for toxicological interactions are direct chemical-chemical, pharmacokinetic, and pharmacodynamic mechanisms.

Chemical-Chemical Interactions. In direct chemical-chemical mechanisms, one chemical directly interacts with another, causing a chemical change in one or more of the compounds. This is the mechanistic basis for many antidotal treatments. Examples of chemical-chemical interactions that result in less-than-additive effects include the use of chelating agents to complex with metal ions and the oral administration of ammonia as an antidote to react with ingested formaldehyde forming hexamethylene-tetramine (Goldstein et al. 1974). An example of chemical-chemical interactions that result in greater-than-additive effects is the formation of carcinogenic nitrosamines in the stomach following ingestion of noncarcinogenic nitrites and amines (Klaassen 1996).

Pharmacokinetic-Based Interactions. Pharmacokinetic-based interactions can be divided into effects on absorption, distribution, metabolism, or excretion. Examples of pharmacokinetic-based interactions are presented in Table 3. The enhancement or reduction of the absorption of one chemical as a result of the presence of another can result from chemical formation of poorly absorbed conjugates or complexes, decreases in gastrointestinal motility, increases or decreases in pulmonary ventilation rate, or disruption of cellular permeability

Interactions that can alter the distribution of a chemical or its metabolite(s) include displacement from an inactive site to a primary receptor site, release from a storage site (e.g., body fat, bone), or competition for plasma protein binding sites. Altered patterns of metabolism are the mechanistic bases for a large number of chemical interactions. The most studied enzyme system involved in interactions is the liver microsomal mixed function oxidases (MFO; cytochrome P450 isozymes). Other enzyme systems that may play a role in interactions include alcohol and aldehyde dehydrogenases, monoamine and diamine oxidases, dehydrochlorinases, azo and nitro reductases, and hydrolases (Mumtaz and Hertzberg 1993). Metabolic interactions can involve induction or inhibition of enzymes. Enzyme induction studies often utilize a sequential pattern of exposure, in which the first chemical causes an induction of specific isozymes and an increased capacity to metabolize other chemicals in the mixture. Simultaneous exposure to a mixture can cause two or more chemicals to compete for enzyme-mediated biotransformation, resulting in an inhibition of the metabolism of at least one of the chemicals. This interaction can result when the chemicals bind to the same active site of the enzyme (competitive inhibition), when a chemical binds to the enzyme and the enzyme-substrate complex (noncompetitive inhibition), or when a chemical binds to the enzyme-substrate complex to form an inactive complex (uncompetitive inhibition) (Krishnan et al. 1994). The fourth type of pharmacokinetic interaction mechanism is alterations in pulmonary, renal, or biliary excretion. In these types of interactions, one chemical can affect the excretion of another by increasing pulmonary clearance, increasing urine alkalinity, vasoconstriction resulting in decreased urinary output, and increasing or decreasing clearance from the liver into the bile.

Table 3. Examples of Pharmacokinetic Interactions		
Basis of interaction	Greater-than-additive effect	Less-than-additive effect
Absorption	Neurotoxicity of EPN enhanced by aliphatic hydrocarbons due in part to increased dermal absorption (Abou-Donia et al. 1985) Increased pulmonary uptake of air contaminants when they are present along with hydrogen cyanide, which at low concentrations increases the pulmonary ventilation rate (Krishnan et al. 1994)	Dietary zinc inhibits some aspects of lead toxicity in part by decreasing dietary lead absorption (Cerklewski and Forbes 1976) Decreased dermal absorption of *m*-xylene and isobutanol due to dehydration of skin elicited by isobutanol (Krishnan et al. 1994)
Distribution	Increased neurotoxicity from increased lead levels in brain after treatment with disulfiram, due to formation of lipid-soluble complexes that can readily distribute to brain (Oskarsson and Lind 1985; Oskarsson et al. 1986a, 1986b)	Selenium protects against cadmium toxicity by decreasing the concentration of cadmium in liver and kidney and by redistributing cadmium in the testes from the low to high molecular weight Cd binding proteins (Chen et al. 1975)
Metabolism	Organophosphorous compounds potentiate the toxicity of fenvalerate and malathion by inhibiting esterase which detoxifies many pyrethroid insecticides and also malathion (Gaughan et al. 1980)	Selenium inhibits 2-acetylamino-fluorene-induced hepatic damage and tumorigenesis in part by shifting metabolism towards detoxification (ring hydroxylation) relative to metabolic activation (Marshall et al. 1979) Ethanol acts as a competitive inhibitor of alcohol dehydrogenases thereby decreasing the metabolism of methanol to formaldehyde and formic acid (Goldstein et al. 1974)
Excretion	Decreased renal excretion of penicillin when co-administered with probencid, potentiating its therapeutic effect (Levine 1973)	Ethanol depresses the conversion of elemental mercury into ionic form resulting in a decrease in pulmonary retention and blood mercury levels and an increase in pulmonary excretion of mercury (Krishnan et al. 1994) Arsenic antagonizes the effects of selenium in part by enhancing the biliary excretion of selenium (Levander and Argrett 1969)

Pharmacodynamic Interactions. The third mechanistic basis for interactions is pharmacodynamic interactions. In pharmacodynamic interactions, the interaction occurs at the cellular receptor site or target molecule, or among receptor sites or targets. Interactions at the same receptor site usually result in inhibitory or antagonistic interactions and are commonly termed receptor antagonism. An example of receptor antagonism is atropine antagonism of organophosphate poisoning by blocking acetylcholine receptor sites (Goldstein et al. 1974; Klaassen 1996). Pharmacodynamic interactions involving binding to different sites on the same molecule can result in greater or lesser toxicity: an example of a synergistic interaction is the synergistic inhibition of inosine monophosphate dehydrogenase activity by tiazofurin and selenazofurin metabolites (Chou and Rideout 1991) and inhibition of copper binding to DNA by other divalent cations (Sagripanti et al. 1991). Interactions among different targets may be functionally antagonistic, as exemplified by the opposing effects of histamine and norepinephrine on vasodilation and blood pressure (Levine 1973). An interaction at different targets that increases toxicity is the potentiation of carbon tetrachloride hepatoxicity by chlordecone inhibition of hepatocellular repair (Mehendale 1994).

General Guidance for Preparing an Interaction Profile

The profiles should be prepared in accordance with the editorial guidelines in Appendix C.

It is appropriate to vary the depth of the presentation in the interaction profile in accordance with the amount of information available on the subject mixture. The profile is intended to present ATSDR's interpretation of the whole mixture and joint toxic action data for use by environmental health scientists. Supporting data and evaluations should be presented in enough detail to allow the reader to understand the conclusions drawn from the study. Thorough evaluations are required.

Consider all data when making conclusions. In general, support all conclusions with references to the original literature. For data-rich profiles, some exceptions may be advisable for less relevant or supporting data, such as *in vitro* data, or for mechanistic data, if voluminous. In these cases, a high quality review that provides an evaluation of the data may be used, and individual studies may be retrieved and consulted as needed for clarification. Refer to an abstract only if the original paper is not obtainable. Older abstracts should be disregarded if not followed up in the literature. Provide explanations when your conclusions differ from those of the study authors or when studies that are widely cited in the literature are not used as the bases for conclusions. The description should be limited to those factors that are necessary to summarize the issue; do not include all the details of the study.

The phrase "ATSDR boilerplate" denotes text to be included in every interaction profile. All such required material is provided in this guidance document. Boilerplate material is presented in a bold type to distinguish it from guidance information.

The term “component mixture” is used in this document to refer to mixtures containing some, but not all, of the chemical constituents of the mixture of concern. For example, a mixture of four chemicals (ABCD) would include component mixtures with three of the four chemicals (ABC, ABD, ACD, BCD) and two of the four chemicals (AB, AC, AD, BC, BD, CD).

OUTLINE FOR AN INTERACTION PROFILE

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Appendix A: Background Information for Chemical A
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Appendix B: Background Information for Chemical B
- *Sections B.1, B.2, B.3, B.4, and B.5 as for Appendix A*

Additional appendices for additional chemicals

SPECIFIC GUIDELINES BY SECTION

This section provides specific guidance for writing an interaction profile. The comments are organized following the outline of an interaction profile.

Title Page

The title page for the interaction profile should be formatted as shown in Exhibit A.

Preface

The preface for the interaction profile should be formatted as shown in Exhibit B.

Summary

The purpose of this section is to concisely present the conclusions and recommendations of the profile up front where the reader can easily find them. Briefly summarize the essential findings regarding the pertinent whole mixture and joint toxic action data, including PBPK or PBPK/PD models. Present the conclusions regarding the relevance of these data to public health, and the recommended approach for exposure-based assessments of potential noncancer or cancer health hazards from waste-site specific (or other) exposure scenarios. Avoid cluttering the summary with string references. References should be cited only as needed for clarity; some summaries will not need to include any citations. The length of the summary should be about 1–4 pages.

Additional guidance regarding the formulation and presentation of conclusions and recommendations is provided in the parts of this guidance document pertaining to Sections 2.3, 3, and 4 of the interaction profiles.

Table of Contents

Generate the table of contents for the interaction profile using the appropriate WordPerfect tool. The table of contents should include all major headings and subheadings down to the level of 2.2.*x*. Regenerate the table of contents after each round of revisions.

List of Abbreviations

Include an alphabetically arranged list of all abbreviations, acronyms and symbols used in the interaction profile with definitions.

1. Introduction

Include the following ATSDR boilerplate regarding purpose of the interaction profile as the first paragraph in this chapter:

> **The primary purpose of this Interaction Profile for [Chemicals X, Y, Z...] is to evaluate data on the toxicology of the "whole" mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture MRL, and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR DT's recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.**

In addition to the above boilerplate, the introduction to the interaction profile should include a discussion of the basis for choosing the mixture of concern. The most likely reasons for selecting a mixture relate to frequency of occurrence, toxicological significance, or some combination of these two factors. Frequency of occurrence may refer to the individual chemicals or to co-occurrence of small groups of chemicals,

with or without consideration of the amounts present and toxicity values. The selection may be based on occurrence at a particular type of site (e.g., waste recycling facilities, mining and smelting sites) and/or in a particular environmental medium (e.g., water, air, or soil). Toxicological significance may refer to toxicity of the individual components or to known or suspected interactions between mixture components.

The rationale for selection of the mixture of concern will be provided by ATSDR, although the contractor may be asked to help with the selection and to write up the description of ATSDR's reasoning. The rationale is significant because it may provide insight into the route and pattern of exposure that are expected to be most important for the mixture of concern. This information could affect interpretation of the interactions data, and in particular, the amount of weight that is given to studies performed by various routes and durations.

As stated previously, the interaction profiles are intended to cover simple mixtures of up to 10 components. Nevertheless, the mixtures selected for some of the first profiles have included complex mixtures as one of the components of the mixture. Inclusion of a complex mixture is possible only if simplifying assumptions can be used, such as treating the complex mixture as a single entity or component (e.g., PCBs) or using the TEF approach for the components of the mixture (CDDs).

2. Joint Toxic Action Data for the Mixture of Concern and Component Mixtures

Purpose and Overview of this Section

This is the main body of the interaction profile. It is here that any relevant whole mixture data and the joint toxic action data are presented and evaluated. The conclusions drawn from evaluation of the data will serve as the basis for the recommendations for the exposure-based assessment of joint toxic action of the mixture. Important considerations for development of this section include: how to locate joint toxic action data, the types of pertinent data that one might encounter, criteria for evaluating joint toxic action studies, the role of PBPK and PBPK/PD models, and the way in which this information should be presented in the profile.

Locating Mixtures and Joint Toxic Action Data

Information on the components of the mixture should be collected as a preliminary measure. This documentation should include not only health effects information, but also toxicokinetic and mechanistic information. The information will be needed for the appendices to the interaction profile and is useful in defining the chemicals to be included in the searches of electronic databases. The major source of this type of information for many of the components will be ATSDR's toxicological profiles. These documents also contain some information on interactions.

The searches of electronic databases are usually performed so as to look for any combination of a discrete set of chemicals, e.g., particular metals (and their compounds), specific solvents, etc. Therefore, as a first step, TOXLINE (1966–2000) is searched for references in which any of the possible pairs of these chemicals occur together in the same reference. For certain chemical combinations, this results in a set of references that can be screened quickly for relevance. However, for many major industrial chemicals, this results in an exceedingly large and diverse set of references ranging from broad scale mutagenicity screening studies to references to site assessments where many chemicals are found, most of which have nothing to do with joint toxic action among chemicals. When this situation occurs, further limit the search by combining the results of the chemical combination search with a set of terms that should reduce the number of retrieved studies to those where some sort of interaction or mixture is studied or noted. These terms range from general terms like "mixture" or "interaction" to specific terms like "synergistic". While many irrelevant studies are still recovered with this search strategy, the strategy does focus the retrieval to more relevant studies. This strategy works best in the bibliographic databases to the more recent literature, since this "keyword" strategy is most effective when there are abstracts as well as titles and indexing keywords to search. The databases to the older literature often consist only of titles and indexing keywords and, only occasionally, brief abstracts. Since TOXLINE is being discontinued in its present form, a similar strategy could be applied in most of the databases, subsets of which have traditionally been a part of TOXLINE (MEDLINE, Biosis, NTIS, etc.), although it may be necessary to adapt the strategy to the particular indexing of each of these databases. Additionally, in the future, the TOXLIT database might also be searched in conjunction with these other databases to balance the coverage of the literature.

Although the MIXTOX database (EPA 1990, 1992) and Integral Search System (ISS) and its supporting databases (Arcos et al. 1988; Bagheri et al. 1988-89; Rao et al. 1989; Woo et al. 1994) are not up-to-date, they do provide a potential valuable source of older interaction studies, and can be searched as needed for

additonal information. Other sources of information on interactions include reviews, but most reviewers focus either on conceptual issues in mixtures health or risk assessment or on specific, rather narrow groups of chemicals (Calabrese 1991; Chou and Rideout 1991; Goldstein et al. 1990; Yang 1994). One exception is a monumental review by Drs. Krishnan and Brodeur (1991). This review at least attempts to encompass much of the literature up to 1990.

Evaluating Joint Toxic Action Studies

To assess potential additivity and interactions that may occur among chemicals in a mixture and the effect that interactions will have on the inherent toxicity of the individual components of the mixture requires a thorough evaluation of the available studies on joint toxic action for the mixture and/or components of the mixture. The studies should be assessed based on the quality of the study and the applicability of the study design to predicting interactions or additivity and potential health outcomes of populations living near hazardous waste sites and exposed to the mixture of concern.

ATSDR has adopted the National Research Council's (NRC's) "Guidelines for Assessing the Quality of Individual Studies," which appear in TOXICITY TESTING: Strategies To Determine Needs and Priorities, published by NRC in 1984. ATSDR agrees with the NRC that judging the quality of past and future studies solely by today's standards is inappropriate. The NRC considers a report of scientific findings adequate for use in health hazard assessment if the report meets the following basic criteria (refer to Appendix A of this guidance document):

- All elements of exposure are clearly described.
- Results in test subjects are predictive of human response, and test subjects are sensitive to the effects of the substance.
- Controls are comparable with test subjects in all respects except the treatment variable.
- Endpoints answer the specific questions addressed in the study, and observed effects are sufficient in number or degree to establish a dose-response relationship that can be used in estimating the hazard to the target species.
- Both the design and the interpretation of the study allow for appropriate statistical analysis of the data.

Where appropriate, these criteria should be applied to judgments on the quality of data from epidemiological investigations and other scientific studies of relevance to ATSDR's toxicological profiles.

The reliability of epidemiological data in hazard identification is increased when results are obtained from studies that have the following characteristics (refer to Appendix B of this guidance document):

- Are derived from well-designed and well-executed case control or cohort studies that are free from bias.
- Display a strong association unlikely to be due to chance variation.
- Follow a logical, temporal sequence of exposure-response.
- Have been replicated in a variety of settings.
- Exhibit a dose-response relationship, using valid estimates of exposure or dose.
- Are toxicologically plausible.
- Where possible, include an examination of causality.

In addition, ATSDR recognizes the following desirable factors of studies or reports of scientific findings as set forth in the NRC guidelines:

- Subjective elements should be minimized.
- Peer review of scientific papers and of reports is desirable.
- Results reported have increased credibility if they are supported by findings from other investigations.
- Similarity of results to those of tests conducted on structurally related compounds increases scientific confidence.
- Evidence of adherence to good laboratory practices improves confidence in results.

Good quality studies designed to assess the possible mode by which *two or more* chemicals affect a biological outcome should include:

- Characterization of the effects of the individual components (and their dose-response relationships) on the outcome.
- Generation of a hypothesis regarding the mode of joint action (e.g., dose-additive, response-additive).

- Prediction of responses to mixtures of the components based on the postulated mode of joint action.
- Observations of the response to mixtures of the components.
- Statistical comparison of the predicted responses with the observed responses to the mixture.

An illustration of an optimal type of study involves two chemicals (A and B) that both individually affect a biological outcome. Dose-response data for each chemical alone indicate that linear dose-response models are adequate to describe dose-response relationships, and that A is 3 times more potent than B. Based on postulated joint additive action in which the null hypothesis is that the two chemicals behave as if they were concentrations or dilutions of one another (dose addition), a mixture of 1 dose unit of A plus 3 dose units B would be predicted to produce a response equivalent to that produced by 2 dose units of A alone, 6 dose units of B alone, or a mixture of ⅔ dose unit of A and 4 dose units of B. If observed responses to the mixture are greater than predicted responses, evidence is provided of a greater-than-additive joint action. Conversely, if observed responses are less than predicted responses, there is evidence of a less-than-additive joint action, as compared with dose addition. More complete discussion of statistical methods (and study design characteristics) to compare predicted and observed responses to mixtures are discussed by Berenbaum (1981), Calabrese (1991), Kodell and Pounds (1991), Mitchell (1986), and Svendsgaard and Hertzberg (1994).

Unfortunately, the toxicological literature on possible interactions among chemicals contains only limited numbers of studies that have all of the features of an optimal joint toxic action study. A standard design that is often followed (2x2 factorial design) involves a zero dose group (control), and chemicals A and B tested alone at doses of A_1 and B_1 and in combination at a dose of A_1+B_1 . This type of design does not provide a full characterization of joint action, and the statistical analysis provided in such studies often provides only information as to which treatment results are significantly different from other treatment results, rather than an indication of whether the results are indicative of a departure from additivity (i.e., an interaction). A problem with this study design is that the mixture group received a higher dose of chemicals than did the single-chemical groups. Thus, any difference in outcome seen in the mixture group may be due to the higher dose rather than to an interaction. Nevertheless, such studies can provide some clues as to mode of joint toxic action; results from such studies can provide evidence of "apparent" greater-than-additive or less-than-additive joint actions (Calabrese 1991). A few examples of types of results and the conclusions that can be drawn are as follows:

- If A_1 produced an increase in response (incidence) of 5% and B_1 produced an increase in response of 10% (as compared with controls), the predicted increase for the mixture of A_1+B_1 would be 15%. If the actual increase from A_1+B_1 was approximately 15%, a tentative conclusion of additivity can be drawn. If the increase from A_1+B_1 was 25%, a tentative conclusion of greater-than-additive action can be drawn, and if the increase was 7%, a tentative conclusion of less-than-additive action can be drawn.

Similarly:

- If A_1 produced an increase of 10 units in a continuous variable (compared with non-exposed controls), and B_1 produced an increase of 20 units in the variable, then the predicted increase for the mixture of A_1+B_1 would be 30 units. If the actual increase was approximately 30 units, a tentative conclusion of additivity can be drawn. If the actual increase from the mixture was 50 units, a tentative conclusion of greater-than-additive action may be drawn, and if the increase was 15 units, a tentative conclusion of less-than-additive action may be drawn.

The above approaches to analyzing the data assume linearity of dose-response. A potential problem with the above study design is that the dose for the mixture group, which is higher than the dose for each the single-chemical group, may lie outside the linear region.

Note that some types of results do not provide the basis for conclusions as to the *mode* of joint toxic action, although they may be useful in assessing potential hazard. An example follows:

- If neither A_1 nor B_1 produced an increase in response or a change in a continuous variable (compared with non-exposed controls), but A_1+B_1 produced an increase in response of 15% or an increase of 15 units in the continuous variable, no conclusion as to the *mode* of joint action can be drawn. The appropriate conclusion is that subthreshold doses of A and B, when given in combination, produced an effect. This conclusion indicates that estimating hazard or risk of the mixture on the basis of each chemical by itself may underestimate the hazard or risk of exposure to the chemicals in combination.

A simple modification of the 2x2 factorial design can help to provide better, albeit still limited, information on the mode of joint toxic action. This design involves a zero dose group, and chemicals A and B tested individually at doses of A_1 and B_1 and in combination at a dose of $\frac{1}{2}A_1$+$\frac{1}{2}B_1$. If the

observed response to the mixture is not significantly different from the predicted response (½ net response to A_1 plus ½ net response to B_1), evidence of additivity is provided. If the net response (or increase in continuous variable) to the mixture is significantly greater (or less) than a predicted sum of ½ net response to A_1 plus ½ net response to B_1, evidence of greater- (or less-) than-additive joint action is provided. This design has the advantage that the dose for the mixture is in the same range as that for the single chemicals. With this design, if both A_1 and B_1 produced no change from control when given separately, but the mixture of $½A_1+½B_1$ produced an increase in response or continuous variable, then a tentative conclusion of greater-than-additive joint action can be drawn.

Additionally, joint toxic action studies should be assessed as to whether the experimental design is relevant for assessing potential health outcomes of populations living near hazardous waste sites. Components of the experimental design that could influence the applicability of joint toxic action studies include exposure route, duration of exposure, sequence of chemical administration, vehicle, dose, and endpoints. Inhalation, oral, and dermal exposure are the most likely routes of exposure for the population of concern, and emphasis should be placed on interaction studies using these exposure routes. In the absence of data for a particular exposure route, data from other exposure routes may be used to predict interactions and health outcomes. Use of data from another route would be based on the assumption that once a chemical has entered the body, there are no route-specific differences in toxicity or potency. However, this assumption may not be true if portal-of-entry effects or first pass effects are expected to occur. First pass effect refers to the metabolism that can take place in the portal of entry tissue, prior to entry into the systemic circulation, and can modulate the dose to remote or systemic target tissues in a route-dependent fashion. First pass effect is usually considered with oral exposure because many chemicals are directly delivered from the gastrointestinal tract to the liver via the portal vein. The respiratory tract can also exhibit a first pass effect after inhalation exposure. Although parenteral exposure is not an exposure route of concern, parenteral administration studies should be reviewed and evaluated if few or no studies using more relevant routes are available, because these data can provide valuable information on potential interactions and can provide mechanistic data. The relevance of parenteral studies to interactions involving oral exposure to the metals, however, needs careful consideration because parenteral administration bypasses homeostatic mechanisms and potential points of interaction related to absorption from the gastrointestinal tract.

Interactions among chemicals in a mixture can vary with duration of exposure. This is particularly true for chemicals that are toxic following chronic exposure but have low acute toxicity, or for chemicals whose biotransformation involves enzyme induction. When reviewing interaction data, the applicability

of the results to different exposure durations should be carefully considered. The toxicity/carcinogenicity and toxicokinetic databases for the chemicals of concern may provide useful information to support or refute extrapolation across exposure durations.

Joint toxic action studies utilize two patterns of administration: simultaneous and sequential. In the simultaneous administration study design, the mixture components are administered at the same or virtually the same time using the same or different exposure routes. As this pattern of administration most closely resembles environmental exposure, greater emphasis should be placed on these data. Prior to 1991, many interaction studies employed a sequential pattern of administration, in which a chemical that alters metabolism or physiology in a known manner was administered before a single dose or exposure of the chemical of concern, in order to investigate the impact on the second chemical's toxicity (Hertzberg and Durkin 1994; Mumtaz and Durkin 1992). This study design provided data useful in elucidating the mechanism of action of the second chemical, but may not be as useful in understanding potential joint toxic action involving low level, long-term simultaneous exposure.

Dose is a very important factor to consider when evaluating interaction studies. In general, an understanding of the dose-response relationships for the individual components of the mixture is important for understanding potential interactions and health outcomes following exposure to the mixture. For example, if the dose tested is much lower than the threshold for the toxic endpoint of concern, then a potential interaction may not be detected by the study. On the other hand, if the dose used is too high, the dose may overwhelm the normal metabolic processes, resulting in different metabolites or an accumulation of a particular metabolite. Similarly, when examining potential interactions for a certain health effect, it is important to examine what other effects are occurring at the tested doses, and, in particular, whether the dose is so high that it is causing serious health effects in other organ systems, or death.

The endpoints of the study also must be evaluated in terms of their relevance to human health and to the known effects or pharmacokinetics of the components of the mixture. Studies that investigate a broad range of endpoints are preferable. Of particularly importance for noncancer effects is the assessment of interactions on the known critical effects of each component, and on relatively sensitive effects in common across two or more components of the mixture.

Role of PBPK and PBPK/PD Models

Physiologically-based modeling of chemical interactions is a promising avenue for mixtures assessment. PBPK models use mathematical descriptions of biological processes together with physiochemical (e.g., tissue:blood partition coefficients), biochemical (e.g., rate constants for metabolism and binding), and physiological (e.g., tissue volumes, blood flow rates, breathing rates) characteristics to model the disposition of a chemical in the body. A PBPK/PD model includes a component to account for the chemical's action in the body (i.e., tissue response to the chemical).

PBPK models for individual chemicals can be joined to model interactions in simple chemical mixtures. The linking of such models is generally performed by investigators who have expertise in PBPK and PBPK/PD modeling and are engaged in laboratory research, either directly or through collaborative efforts, to validate the models. PBPK models are available for several binary mixtures and a few mixtures with three or more components. A PBPK model for a binary mixture is constructed by joining PBPK models on the individual constituents at the point of interaction. For most of the models that have been developed to date, this means the hepatic metabolism term. For example, if it is known that both chemicals are substrates for a specific isozyme of cytochrome P450, it might be hypothesized that the chemicals would interact by competitive metabolic inhibition. The models for the individual chemicals are joined at the metabolism term and the assumption of competitive inhibition is tested. Once the assumed mechanism of interaction is validated by comparing model predictions with experimental data for administration of the chemicals alone and together, the model can be used to predict effects of co-exposure for different exposure scenarios.

PBPK models for higher order mixtures can be derived by adding models for individual chemicals to the existing interactions model. Each time a new chemical is added, it is linked to the models for the component chemicals based on the pairwise interactions mechanisms. Therefore, if chemicals A and B have models that are joined at the point of interaction and chemical C is added to the mixture, chemical C is added to the model in two places—its point of interaction with chemical A and its point of interaction with chemical B. The effect of C on the interaction between A and B is automatically simulated in the model because all components are linked with each other within the PBPK framework. In this way, complex PBPK models for higher order mixtures can be built based only on knowledge of the binary interaction mechanisms among the component chemicals (Haddad and Krishnan 1998).

PBPK models provide a powerful tool to investigate hypotheses regarding mechanisms of interaction between chemicals. They can also be used to identify apparent thresholds for interactions and potentially to extrapolate across dose, duration, sequence, route, and species. If the model has a pharmacodynamic

component, it can be used to predict toxicity (tissue response) in addition to pharmacokinetic interactions, and the interactions that can be modeled would expand to include those where the tissue response per unit dose is altered, in addition to those where the dose delivered to the tissue is changed.

Because PBPK and PBPK/PD models are such a potentially powerful tool for the analysis of chemical mixtures, it is important to describe any PBPK models available for the mixture of concern or its component mixtures in the interaction profile. Evaluate how the models are linked and the relevance of the modeled tissue dosimetry or response to the toxicity endpoints and exposure scenario of concern for humans. Consider whether the validation of the models was adequate. Examples of linked models that have some applicability to assessment of potential health hazard from exposure to chemical mixtures are:

- PBPK/PD modeling to estimate levels of carboxyhemoglobin in humans exposed for 8 hours to threshold limit value (TLV) concentrations of dichloromethane and toluene, as compared with dichloromethane alone (Pelekis and Krishnan 1997). Although the investigators had a different purpose for conducting this PBPK/PD simulation, the approach illustrates the use of PBPK/PD modeling to estimate the effect of co-exposure to toluene on the induction of carboxyhemoglobinemia (adverse effect) by dichloromethane in humans at defined levels of exposure, and to identify apparent thresholds for interaction.
- A PBPK model for acute exposure to benzene, toluene, ethylbenzene, and *m*-xylene that predicts blood concentrations of the parent compounds in rats following inhalation exposure to the mixture (Haddad et al. 1999a). This model has not yet been adapted for application to humans. Blood levels of the parent compounds may be relevant to central nervous system effects.
- Use of separate and linked PBPK models to estimate biological hazard indexes (HIs; based on blood concentrations of parent compound) for varying exposures and proportions of a three-chemical mixture (toluene, ethylbenzene, and *m*-xylene) (Haddad et al. 1999b). (These biological HIs may be relevant to the central nervous system effects of the compounds.)

Presenting Joint Toxic Action Information in the Profile

The level of detail of the presentation of joint toxic action data should be directed at describing the mechanism and toxic consequences of the joint action. Important details might include species, route, duration, sequence, relative proportion of chemicals in the mixture, and whether or not a threshold was demonstrated. The description should be sufficient to support the evaluation of the data, particularly with regard to applicability to human exposure at hazardous waste sites (presumably low level, repeated or

chronic, simultaneous exposure). Complete study descriptions may not be not necessary. As a general rule, experimental details should be included only to the extent that they are needed to address specific issues with respect to the joint toxic action data and to explain and support the conclusions drawn from the study.

General guidance for organizing the evaluation of joint toxic action data are provided in this paragraph. There may be data sets that are best discussed with a different organization; involve the ATSDR profile manager in any decision to depart from the recommended organization. When human data are available, these data should be presented before the animal data on joint toxic action. The types of human data which are likely to be available include epidemiology studies, clinical studies or controlled exposure experiments, and case reports. High quality epidemiology and clinical/experimental studies may be more useful for assessing joint toxic action than case reports because a larger number of subjects were examined, a control group is often used, the exposure assessment may be more complete, and appropriate statistical methods may have been used to detect main and interaction effects. Because case reports can provide some useful qualitative information, these data should not be overlooked and should be briefly discussed after the epidemiology and clinical/experimental studies. The discussion of animal data should follow the discussion of human data. Animal studies that use inhalation, oral, or dermal exposure routes should be discussed prior to parenteral administration studies, and studies examining toxicity or carcinogenicity endpoints should be discussed first, followed by studies that focused on mechanistic alterations. Depending on the quality of the available data base, and the usefulness for mechanistic analysis, *in vitro* studies may be included; they should be discussed after the *in vivo* animal studies.

2.1. Mixture of Concern

Discuss any data or models that were located regarding the actual mixture of concern or similar mixtures. A *similar* mixture is one that has the same chemicals as the mixture of concern, but in slightly different proportions, or one that has mostly the same components in highly similar proportions. Similar mixtures are expected to have similar fate, transport, and health effects as the mixture of concern. Data on a sufficiently similar mixture are treated as if they were collected using the mixture of concern.

The discussion should follow the general guidelines provided above regarding evaluation and presentation of joint toxic action studies. Keep in mind the relevance to human exposure scenarios when interpreting the data. For example, for simple mixtures at hazardous waste sites, the most relevant data would be for low level, repeated or chronic, simultaneous oral or inhalation exposure in humans.

When developing this section, pay particular attention to studies that may be adequate to serve as the basis for an MRL value for the mixture of concern. Such studies are rarely available, but would ideally include a range of dose levels for the whole mixture and different proportions of the components, or justification of the appropriateness of the tested mixture as the mixture of concern for the actual exposure scenario. The study ideally would assess a wide range of endpoints, including the known critical or sensitive effects of each component and also effects in common across several components. Also pay particular attention to studies that assess the toxicity of the mixture in comparison with each constituent alone or in pairs (as discussed in more detail below, pairs are the most basic unit for the study of interactions). While studies without this comparison can provide an indication of the toxicity of the mixture that would include any interactions occurring between components, studies with such a comparison can also provide evidence as to whether interactions are actually occurring or whether the observed effect is simply the result of additive or independent action of the components. Studies with this comparison could also identify joint action leading to shifts in critical target organ as more chemicals are added to the mixture.

2.2. Component Mixtures

Purpose and General Instructions for this Section

The purpose of this section of the profile is to provide a critical review of the joint toxic action data on the component mixtures. Unless you know in advance that the assessment for a particular mixture will be based on whole mixture data for the mixture of concern, it will be necessary to analyze the interactions among chemicals in the mixture in terms of the component mixtures. Two-component, or *binary*, mixtures are of special interest. These are the simplest mixtures, and therefore, represent the most promising case for investigation of joint action of chemicals. For this reason, most interaction studies to date have been conducted on binary mixtures.

Pertinent data on component mixtures also may be available from multi-component experimental or epidemiological studies, even if the "mixture" also contains chemicals that are not present in the mixture of concern, provided multivariate analysis has been used, and the study was adequately designed.

A mixture of N chemicals will have $(N^2-N)/2$ component pairs. This means that a mixture of 3 chemicals will contain only 3 component pairs, but the number of pairs will increase rapidly with increasing size of the mixture, so that a mixture of 5 chemicals will contain 10 component pairs and a mixture of

10 chemicals will contain 45 component pairs. Therefore, while component-pair interactions provide a useful basis for predicting the interactions in simple mixtures, the feasibility of using the pairwise approach drops quickly as the size of the mixture increases.

Interaction profiles are developed for simple mixtures of no more than 10 chemicals. For such mixtures, the pairwise assessments often may form the basic units of the mixtures assessment. Each binary mixture should be discussed in its own numbered section. All relevant information for the pair should be discussed in this section, including joint toxic action data, joint toxicokinetic data, known or potential mechanisms of interaction, and any existing PBPK and PBPK/PD models for the mixture. Pairwise studies generally include administration of the individual components as well as the mixture, and if properly designed (see above section on evaluating joint toxic action studies), the results will be able to distinguish between additivity (or no influence) and interactions. If data are voluminous, separate sections may be broken out for discussion of joint toxic action and joint toxicokinetics, and these sections may further be broken down based on endpoint. If data for a pair are not available, information regarding joint action of structurally or functionally related chemicals can be used. In the absence of toxicologic or mechanistic joint action data, it is appropriate to consider the potential for interactions or additivity between the two chemicals based on their background toxicity, mechanism of action and toxicokinetics data. Available data for each pair are summarized in a table, such as that shown in Exhibit C. Directions for preparing such a table are provided below.

The discussion should follow the general guidelines provided above regarding evaluation and presentation of joint toxic action studies. Keep in mind the relevance to human exposure scenarios when interpreting the data. Studies that most closely match the expected human exposure conditions should carry the greatest weight. If available, PBPK and PBPK/PD models for the mixture should be described and evaluated. The role of these models has been described previously. Evaluate how the models are linked and the relevance of the modeled tissue dosimetry or response to the toxicity endpoints and exposure scenario of concern for humans. Consider whether the validation of the models was adequate.

When data are available for component mixtures larger than pairs, these data should be presented in numbered sections (one per component mixture) *preceding* the sections for binary mixtures. Only include sections for the 3-or-more component mixtures for which joint action data and/or models actually exist.

Depending on how the mixtures of concern are chosen by ATSDR, there may be overlap in chemical constituents of the mixtures evaluated in interaction profiles. Overlap of more than one chemical constituent will lead to overlap of component mixtures, as well. Prior to starting work on the interaction profile, the contractor must find out from ATSDR if any of the component mixtures have been included in previously completed interaction profiles. If so, the existing assessments on these component mixtures should be obtained from ATSDR. Existing component mixture assessments should be updated (if more than 3 years old) and used in the new interaction profile.

*Preparation of Summary Table*s

Each interaction profile should contain a series of tables that summarize the available joint action data for the component binary mixtures. The summary tables provide an overview of the influence of each chemical on the toxicity/carcinogenicity of the other chemical in the mixture. Each binary mixture is described by two sets of two summary tables. One set of summary tables includes studies with simultaneous exposure data, while the other includes studies with sequential exposure data. The sequential exposure data are separated from the simultaneous exposure data because they are considered less relevant to exposure scenarios for chemicals at hazardous waste sites. For both simultaneous and sequential exposure, there are two tables: one for the influence of chemical A on chemical B and one for the influence of chemical B on chemical A. A sample set of summary tables for simultaneous exposure is presented in Exhibit C. Studies using relevant routes of exposure (inhalation, oral, dermal) are higher priority for inclusion in the tables. The summary tables are organized by route (inhalation, oral, dermal) and duration (acute, intermediate, chronic) of exposure. Only tables with data should be included in the profile, and within each table, only the routes and durations with data should be included.

Each target organ/endpoint with data for a given exposure route and duration is listed in a separate row box. Within a row box (specific endpoint for a given route and duration), each study with relevant data is listed in its own row, with the result in the appropriate interaction column (greater than additive, additive, less than additive) described by dose for chemical A + dose for chemical B with species code in parenthesis, and the corresponding reference given in the same row in the reference column. Within a row box, the studies (rows) are arranged by species (rat, mouse, etc.), then dose of chemical A (low to high), and dose of chemical B (low to high). These instructions apply specifically to the table regarding the influence of chemical A on chemical B; the roles of chemicals A and B are reversed in the table for the influence of chemical B on chemical A. Pertinent toxicokinetics data can be included in these tables,

or placed in separate tables (again for the effect of chemical A on chemical B, and B on A) if the joint action data are voluminous.

Exposure levels are reported using the same units for all studies by a particular route. The units are indicated in parentheses after the route title in the table. Dose conversions (e.g., from dietary concentrations to dose in mg/kg/day) can be explained in the text, or in a separate memorandum if there are so many that the flow of the document would be disrupted by their inclusion. These conversions are performed as for a toxicological profile.

If a single study includes multiple doses of chemical A and/or B, then indicate ranges instead of single doses in the appropriate results column. If a multiple dose study shows a dose-related interaction effect (for example, additivity at low doses and greater than additivity at high doses), then indicate both low- and high-dose outcomes in the appropriate results columns in the same row (in this example, put the low dose range in the additive column and the high dose range in the greater-than-additive column in the same row).

If the two chemicals were administered by different routes, list the results under the route for the chemical whose toxicity/carcinogenicity is being described in the table, and note the exposure route for the other chemical. For example, in the table for the influence of chemical A on the toxicity of chemical B, if chemical A is administered to mice via inhalation exposure and chemical B is administered via oral exposure, the results should be summarized under oral exposure and the entry would be: 100 ppm inhal + 50 (m). Reverse the process for the toxicity of chemical B.

Each row box (specific endpoint for a given route and duration of exposure) has a conclusion column. In the conclusion column, indicate whether the interaction for this route, duration, and endpoint is greater than additive, additive, less than additive, or whether the data are insufficient to make a determination. This conclusion is based on a weight of evidence assessment of the results presented in that row box. If mixed results have been found, this should be very briefly discussed in the conclusion column. For example, a conclusion for an endpoint with mixed results might be:

in general additive, >additive if high doses of chemical A given

2.3. Relevance of Joint Toxic Action Data and Approaches to Public Health

Purpose and Overview of Section

The previous parts of Section 2 presented the joint toxic action data and models available for the mixture of concern and its component mixtures. This section presents a synthesis of the data and an evaluation of the overall data base and conclusions regarding the potential health effects of the mixture, mode of joint toxic action of the components, direction of demonstrated interactions, and, when possible, exposure levels at which these interactions may occur. When data regarding the mixture of concern or joint toxic action of component mixtures are not available or not conclusive, the mode of joint action may be evaluated or postulated based on information for the individual components.

There are several methods available to evaluate or postulate the mode of joint toxic action for chemical mixtures. The most relevant methods for simple mixtures are the use of PBPK and PBPK/PD models and the WOE methodology. Another method that may, following additional development, be recommended is the ISS. Each of these evaluation methods is described below. By using one or more of these methods, it should be possible to arrive at a conclusion regarding the mode of joint toxic action for the mixture of concern and the potential impact on public health. These conclusions will form the basis for recommendations (presented in the portion of this guidance concerning Section 3 of the interaction profiles) to environmental health scientists as to how to incorporate concerns about joint toxic action into site assessments of chemical mixtures.

PBPK and PBPK/PD Models

The development of PBPK and PBPK/PD models for mixtures and the role such models can play in mixtures health assessment were discussed above in the overview to Section 2. These models provide a powerful tool for assessing the joint toxic action of chemical mixtures. They can be used to identify functional thresholds for interactions and potentially to extrapolate across dose, duration, sequence, route, and species. If the model has a pharmacodynamic component, it can be used to predict toxicity (tissue response) in addition to pharmacokinetic interactions, and the interactions that can be modeled would grow to include those where the tissue response per unit dose is altered in addition to those where the dose delivered to the tissue is changed.

In developing the interaction profile, the literature search will identify any PBPK or PBPK/PD models available for the mixture of concern or component mixtures. The author of the interaction profile should report the availability of these models in the profile, describe the specific models available and the conclusions drawn from simulations conducted by the models' developers, and discuss other potential uses of the models for assessment of health effects associated with the mixture of concern. In most cases,

the model developers will have run and reported on simulations that would be applicable towards assessment of the mixture. For example, they might have identified apparent thresholds for the modeled interactions. In this case, a conclusion might be that interactions leading to greater-than-additive hazard or risk occur only above a certain exposure level (which would be reported), and furthermore that the exposure level is not likely to occur through exposure at hazardous waste sites. In this way, the results of model simulations available in the literature could lead to conclusions relevant to the site-specific assessment of joint toxic action for the mixture.

There may also be cases where the model may be directly useful in conjunction with measured exposure levels at the site. For example, if a fully developed PBPK/PD model for the mixture of concern were located in the literature search, it might be possible to use the model to predict the mode of joint toxic action and potential toxic response in humans exposed to the mixture by a relevant exposure scenario (e.g., simultaneous, low level, chronic, drinking water exposure). Direct use of the model could be investigated by consulting with an ATSDR DT PBPK expert regarding the feasibility of ATSDR acquisition and use of model with site-specific data in order to predict potential health consequences of exposure to the mixture at the estimated exposure levels. Discuss this possibility with the ATSDR profile manager during profile development and proceed accordingly. Although such models are not widely available at present, it is anticipated that they will become more common in the future and that ATSDR may provide technical support for the use of recommended models.

The more usual case will be that PBPK models are available for one or more of the component pairs. These models can be used a part of a weight-of-evidence approach, discussed in the following section.

Regardless of how a PBPK or PBPK/PD model is used in the interaction profile, it is important to point out uncertainties associated with use of the model and the potential impact of these uncertainties on the conclusions drawn from the model. Additional discussion of the use of PBPK models in mixtures assessment can be found in ATSDR (2001).

WOE Methodology

In the WOE methodology (Mumtaz and Durkin 1992; Mumtaz et al. 1994), a qualitative binary weight-of-evidence classification (BINWOE) is determined for the effect of each chemical on the toxicity of each other chemical in the mixture. Two BINWOEs are derived for each pair of chemicals in the mixture (one for the effect of chemical A on the toxicity of chemical B and the other for the effect of B on the toxicity

of A). Therefore, interactions in a mixture of 4 chemicals that has 6 component pairs will be characterized by 12 BINWOEs. In the original quantitative WOE method, these BINWOEs were used as interaction terms in an algorithm that adjusted the hazard index for interactions. Because there have been problems with the algorithm (ATSDR 2001), a qualitative WOE method is to used to estimate the impact of interactions on the potential health hazard.

In the qualitative WOE method, a matrix of the BINWOE scores is constructed, as shown in Exhibit D. The matrix is then inspected for any patterns that might be evident. For example, if the BINWOEs all indicate additive or less-than-additive joint action, the conclusion would be that the mixture evidenced little potential for interactions that would increase the health hazard or risk beyond what would be expected based on additivity of the components. If the matrix indicated that interactions between chemicals A and B were primarily greater than additive, but chemical C produced less-than-additive interactions with both A and B, the conclusion might be that the mixture possessed potential for greater-than-additive joint action at sites where C is found at much lower levels than A and B. The procedures in ATSDR (2001) eliminate chemicals found at low exposure levels (relative to MRLs or comparable health guidelines) from further consideration of joint toxic action. Therefore, for mixed patterns of BINWOE scores, and for mixtures with similar hazard quotients (ratios of exposures to MRLs or other health guidelines), a further application of the qualitative WOE is to sum the numerical BINWOE scores to estimate a combined score. Professional judgment is used in the interpretation of the impact of the WOE on the hazard index. If the combined WOE score is positive and significantly greater than zero, the conclusion would be that the hazard is likely to be greater than estimated by the hazard index. Conversely, if the combined WOE score is negative and significantly less than zero, the conclusion would be that the hazard is likely to be less than estimated by the hazard index. Combined scores near zero indicate that the hazard index can be used as a reasonable estimate.

The WOE methodology was developed, and has traditionally been applied, for noncancer effects, but it also is applicable to carcinogenic effects.

Binary Weight-of-Evidence (BINWOE) Determinations

A BINWOE determination is an alphanumeric classification intended to characterize how two chemicals are likely to interact. It is a qualitative weight-of-evidence judgment, based on empirical observations and mechanistic considerations, which categorizes the most plausible nature of any potential influence of one chemical on the toxicity of another for a given exposure scenario (Durkin 1995). BINWOEs are

intended to encompass and describe differences in exposure conditions, but not the magnitude of the interaction, and are an attempt to describe the relative concern for interactions much in the same way that fixed uncertainty factors are an attempt to describe relative concern for uncertainties in single chemical assessment.

As summarized in Table 4, a BINWOE indicates the expected direction of a binary interaction (additive, greater than additive, less than additive, or indeterminate), and qualitatively groups the supporting data using a scheme based on two major criteria (mechanistic understanding and toxicological significance) and three modifying categories (duration/sequence of exposure, *in vivo*/*in vitro* data, and route of exposure). The BINWOE scheme can yield a classification of a six-component rating for each pair of chemicals. For example (Mumtaz and Durkin 1992), a rating of >II.A.2.a.ii indicates that the two chemicals may be expected to show greater-than-additive toxicity (>), and that the assessment is supported by the direct observation of a toxicologically significant interaction between the two chemicals and by mechanistic data based at least partly on analogy to other chemicals (II.A). Confidence in this assessment is reduced because, as indicated by the modifying rating (2.a.ii), the supporting data (although *in vivo*) are based on a duration or sequence and route of exposure different from that on which the assessment of interaction is based.

The BINWOE scheme assigns a higher degree of confidence to classifications with low category designations. For example (Mumtaz and Durkin 1992), any classification starting with "II" has a higher level of confidence than one starting with "III", and a "II.A" rating has a higher confidence level than a

Table 4. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

Classification		Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	–1
?	Indeterminate	0
Quality of the Data		**Weighting**
Mechanistic Understanding		
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
Toxicological Significance		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
Modifiers		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05

BINWOE = Direction Factor x Weighting Factor: Ranges from !1 through 0 to +1

*Adapted from Mumtaz and Durkin (1992) and Mumtaz et al. (1994)

"II.B". Due to the data simplification inherent in the scheme, each classification must be accompanied by a brief narrative describing the considerations used to determine the BINWOE.

BINWOEs can be converted to a numerical score for application to site-specific assessments of joint toxic action of chemical mixtures. As discussed by Mumtaz and Durkin (1992), conversion to a numerical score involves converting the BINWOE rating to a direction factor that describes the nature of the likely interaction for the chemical pair and a data quality weighting factor that express confidence in the qualitative assessment of the interaction. The numerical weighting values reflect judgment as to the relative importance of the data quality classifications in determining the weight of evidence. (See Appendix B of the Mixtures Guidance Manual if additional explanation is desired.) The weighting factor is the product of individual data quality weighting scores. The product of the direction and weighting factors can then be used as surrogate measures of confidence in the interaction in order to incorporate concerns about joint toxic action into a site assessment. The qualitative ratings are shown in the left column of Table 4 and the direction and individual data quality weighting scores are shown in the far right column. Using the rating example of >II.A.2.a.ii given above, the corresponding BINWOE score is +1(0.71)(1)(0.79)(1)(0.79) = +0.44. The maximum and minimum possible weighting scores are 1.0 and 0.05. BINWOE scores range from -1 through 0 to +1. The first two components of the BINWOE, mechanistic understanding and toxicological significance of the available data, are primary scores, and as such are the main criteria and the only necessary criteria for developing BINWOEs.

Guidance on determining BINWOE ratings is provided below. A format for presenting the rationale for the BINWOE determinations is presented in Exhibit E. The BINWOE determinations should be preceded by a table presenting the BINWOE classification scheme (Exhibit F).

General Guidance for Determining BINWOEs. The BINWOE determinations focus on the question "if conditions are such that an interaction occurs, what is the direction of interaction likely to be?" This application of the method was specified by its developers (Durkin 1995; Mumtaz and Durkin 1992). For well-studied binary mixtures, the data may suggest no interactions at low doses (joint action appears additive), but interactions at higher doses. In general, the rating should reflect the interaction. Dose is taken into account in the general strategy in ATSDR (2001), which recommends that chemicals present at very low exposure levels relative to an MRL (or comparable health guideline value) be eliminated from further consideration. An exception to the practice of not explicitly considering dose-response data should be made when the data indicate a qualitative change in the direction of the interaction with changes in the dose of one of the chemicals. For example, if chemical A appears to antagonize chemical

B at low doses, but synergize chemical B at high doses, this should be emphasized in the narrative, and, in most cases, the BINWOE should be declined or two separate dose-dependent BINWOEs should be developed. This kind of interaction is unusual, however, and should not be confused with cases in which chemical A inhibits the effects of chemical B, but chemical B enhances the effects of chemical A, which may be attributed to different interactions operating by different mechanisms of action (Durkin 1995). The BINWOE methodology encompasses such interactions by requiring separate BINWOEs, one for the effect of chemical A on chemical B and the other for the effect of chemical B on chemical A. When the direction of the interactions differs, it is usually additivity in one case and some form of interaction in the other (Mumtaz and Durkin 1992).

Differences in experimental conditions are considered because each BINWOE is specific to a given exposure scenario. In particular, the modifying factors are used to express how well the available data correspond to the conditions of the site-related human exposure scenario with respect to duration, sequence and route of exposure. The modifiers are used when the mechanistic and toxicological ratings do not account for the additional concerns for differences in duration, sequence, bioassay (*in vitro* versus *in vivo*), or route of exposure between the site-specific exposures and the data used for the BINWOE determinations. Other experimental dissimilarities, such as species or vehicles, are not listed in the BINWOE classification scheme, but are taken into account during the process of determining mechanistic understanding and toxicological significance. Because ATSDR is most concerned with simultaneous exposures, the BINWOEs should focus on simultaneous rather than sequential exposures. Further discussion of the sequential issue is provided in the following section on modifiers.

Direction of Interaction. Following evaluation of the data, the direction of interaction is classified to indicate whether the type of joint toxic action is additive (=), greater than additive (>), less than additive (<), or indeterminate (?). As discussed by Mumtaz and Durkin (1992), additive is used to represent joint toxic action that is additive by a defined model of additivity (e.g., dose or response addition) and cases in which one chemical does not affect the toxicity of another (no apparent interaction). The "greater than additive" category is used to refer synergism or potentiation. The "less than additive" category applies to antagonism, inhibition, or masking. The "indeterminate" category is applied to instances of ambiguous, conflicting, or no data. Assignment to this category means the direction of interaction cannot be classified, and therefore, no weight-of-evidence determination can be made. Document the reasons for the indeterminate classification, but do not proceed with classification of mechanistic understanding and toxicological significance.

Mechanistic Understanding. This category is used to broadly refer to information concerning the ways in which a chemical causes a given toxic effect or ways in which chemicals interact, and encompasses terms such as "mechanistic information" and "mechanisms of action". As discussed subsequently under Toxicological Significance, the most relevant toxic effects for noncancer assessment are the critical effects of the components of the mixture and sensitive effects in common for the components. Because all of the pharmacokinetic processes that affect the toxicity of a single chemical may be important in joint toxic action, information regarding these processes are encompassed by this primary rating (Durkin 1995). If there is information suggesting that one chemical will affect the absorption, distribution, metabolism, or elimination of another compound and that the effect(s) may be plausibly related to a toxicologically significant response, this information should be incorporated into a mechanistic rating. This information may include linked PBPK models. This category should also include information on events occurring at the molecular or receptor site level and at higher levels of biochemical, physiological, or pathogenic activities, such as morphological or functional effects on various enzymes, organs or systems, that may be related to a toxicological response in the whole animal (Durkin 1995; Mumtaz and Durkin 1992).

The three classifications in the mechanistic understanding category (direct, indirect, and inadequate or ambiguous data) are used to reflect the quality of the mechanistic information supporting the assumption or observation of a toxicological interaction, and the extent to which this information can be interpreted relative to the direction of the interaction (Mumtaz and Durkin 1992). Evidence for the first classification, "Direct and Unambiguous Mechanistic Data," should be interpreted within the broad context described in the previous paragraph. This category is not restricted to cases in which the mechanism of the joint toxic action is fully understood and empirical data are available demonstrating the mechanism (Durkin 1995). For example, if it is known that chemical A inhibits the binding of chemical B to a critical receptor site, this is obviously direct mechanistic information. Also, if it is known that chemical A induces an enzyme that metabolizes chemical B to a more or less toxic agent, this is also considered direct mechanistic information, even if there are no studies demonstrating that chemical A actually influenced the metabolism of chemical B. The evaluation, however, should consider that there may be additional points of interaction. For example, chemical A may induce an enzyme that activates chemical B to a more toxic agent, as well as enzymes involved in the detoxification of that toxic agent. Or chemical A may induce a binding protein that generally sequesters and mitigates the toxicity of chemical B, but also increases its distribution and release to the critical target organ. Therefore, relatively few interactions may qualify for the first classification.

The second classification, "Mechanistic Data on Related Compounds," applies to mixtures in which the mechanism and direction of interaction are not well characterized but can be inferred using qualitative, quantitative or informal structure-activity or chemical class relationships (Mumtaz and Durkin 1992). In addition, if the mechanistic information is for the components of the mixture, but is for endpoints other than the endpoints of concern, the second classification may be more appropriate. A "II" rating is best applied to chemical classes that have been examined extensively for mechanistic similarities and to chemical classes for which common mechanisms of action are known, but for which detailed toxicological studies of many class members are not available. The chemical classes may be obvious structural analogues (e.g., dioxins or PCBs), classes based on common effects (e.g., peripheral neurotoxic agents), or classes based on common pathways and consequences (toxification/detoxification) of metabolism. The extent to which structural or class associations may increase the uncertainty of the assessment will depend on the strength of the available data supporting the association. Although the BINWOE method does not finely differentiate the quality of the data supporting the association, it is necessary to judge whether or not any available information on structurally related chemicals is good enough to influence the nominal BINWOE rating. For example (Mumtaz and Durkin 1992), for chemical classes that have been well characterized, the strength of the structure-activity relationships may be sufficiently strong to warrant an increase to rating "I". Conversely, for poorly documented or more tenuous analogies, a rating of III may be more appropriate. The basis for a rating change and any concerns regarding the use of structure-activity relationships should be articulated as clearly as possible in the narrative supporting each BINWOE.

The third classification, "Inadequate or Ambiguous Mechanistic Data", is actually two distinct categories that have been combined and judged to have the same effect on the BINWOE (Durkin 1995). Rating "III" includes cases in which reasons for the occurrence of the interactions are simply not apparent based on the known characteristics and mechanisms of the individual compounds. Interactions that have been observed or postulated, but for which the quality of the supporting mechanistic information is poor, should be regarded as having inadequate mechanistic data. Mumtaz and Durkin (1992) recommended placing less credence in purely empirical observations of toxic interactions or assumed interactions in the absence of strong mechanistic data when the empirical observation was by a different route and duration than the route and duration of concern. For interactions that are conjectural, the lack of strong mechanistic data greatly reduces confidence in the assessment.

The "III" rating is also used for chemicals with ambiguous mechanistic data, i.e., for chemicals for which there are various types of mechanistic data, including reasonably well documented and consistent

information on mechanisms of action, that can lead to conflicting interpretations of the likely direction of the interaction (Durkin 1995). Examples of this situation, mentioned previously in the discussion of why few mechanistic interactions may qualify for the classification of "direct and unambiguous", include the following: chemical A may induce an enzyme that activates chemical B to a more toxic agent, as well as enzymes involved in the detoxification of that toxic agent. Or chemical A may induce a binding protein that generally sequesters and mitigates the toxicity of chemical B, but increases its distribution and release to the critical target organ. When such ambiguities cannot be resolved, the "III" mechanistic rating should be used. The rationale for a judgment of ambiguous (or inadequate) mechanistic data, including the nature of the ambiguities, should be incorporated into the narrative.

Toxicological Significance. The "toxicological significance" rating (A, B, or C) is used to express confidence that the chemicals will interact in a way that will have a significant impact on the health of the exposed population (Mumtaz and Durkin 1992). Care must be exercised in how interactions are assessed for the toxicological significance. Studies of joint toxic action should explicitly describe the criteria or models used to evaluate whether the results indicate additivity or interactions. Simple statistical comparisons among groups are not necessarily adequate to reveal the mode of joint toxic action, and the study authors' conclusions should not be taken at face value. (See the previous section on evaluating joint toxic action studies.) Even the common type of interaction study that simply evaluates how one chemical affects the toxicity of another should be evaluated to ensure that the non-effective chemical has been shown to be non-effective, preferably in the same study, or at least in other studies. Information from linked PBPK/PD or PBPK models on the binary mixture also may be useful for this rating.

The highest rating, directly demonstrated toxicological significance (A), is intended for instances in which the joint toxic action has been observed directly and is linked to a toxicologically significant endpoint. Although the earlier publications of the WOE method did not discuss the need for this rating to take into account target organ (Durkin 1995; Mumtaz and Durkin 1992), experience in application of the WOE method has indicated that the WOE evaluations should be target-organ specific (ATSDR interaction profiles; Mumtaz et al. 1998). For assessing impact on public health, the relevance of the data to humans must be considered. For noncancer effects, which will be assessed using the hazard index, in some instances with the target-organ toxicity dose (TTD) modification, and through the qualitative WOE, the most toxicologically significant endpoints are the critical effects of one or both of the components and relatively sensitive effects common to two or more components of the mixture. The exposure route and duration in the study in which these effects were observed may or may not be the exposure route or

duration of concern. Depending on the supporting mechanistic information, differences in exposure route or duration can be reflected in the rating for toxicological significance or in one of the modifying ratings discussed below.

A "B" toxicological significance rating is applied to inferred toxicological significance, or the demonstration of toxicologically significant interactions from structurally related compounds (Mumtaz and Durkin 1992). This rating is intended for cases when the interaction can be related to a change in toxicity for the target organs of concern, but no toxic effects were observed (Mumtaz and Durkin 1992). This rating may be appropriate when the toxic effects were for a different target organ than the target organ of concern, but mechanistic data support the relevance, or when confidence in the study is lowered due to limitations in study design, reporting, or data analysis. It may be applied to chemicals that are known to affect the metabolism, absorption, or elimination of one another (Mumtaz and Durkin 1992). The issues associated with interpreting data on related compounds are essentially the same as those described above for the use of structure-activity relationships or chemical classes in assessing mechanistic understanding.

The classifications of inferred toxicological significance and mechanistic data overlap. For example (Durkin 1995), if it is known that chemical A induces an enzyme that metabolizes chemical B to a more or less toxic agent, this is considered direct mechanistic information, even if there are no studies demonstrating that chemical A actually influences the metabolism of chemical B. If such information is available (i.e., data showing that chemical A enhances the conversion of chemical B to a more or less toxic metabolite), it can be treated as an inference of a toxicologically significant interaction, resulting in the same information being used twice (i.e., in the assessment of mechanistic understanding and in the demonstration of toxicological significance). This kind of cross-use of information is appropriate because the understanding of the mechanism by which an interaction may occur and the demonstration that the mechanism is operative for two chemicals enhances the confidence that such an interaction will have toxicological significance.

A "C" rating is used when the toxicological significance of the interaction is not clear. For example (Mumtaz and Durkin 1992), although mechanistic considerations can suggest that an interaction may occur, the toxicological significance of this interaction would be questionable if it has not been demonstrated or cannot be inferred by analogy. This rating is most likely to be applied to assessments based on poorly validated *in vitro* assays or tenuous extensions of mechanistic information. It may also

be appropriate when a direction of interaction has been based on the study thought to be most relevant or reliable, but other studies provide conflicting information.

Modifiers. Each of the modifying ratings is intended to be independent of the mechanistic and toxicological ratings and the other modifying ratings, and can significantly influence the weight-of-evidence assessment for an interaction (Mumtaz and Durkin 1992). An observed interaction may be altered by the duration and sequence of exposures, particularly for chemicals which form chemical complexes (e.g., cadmium and selenium), or exert their interactive influence by the induction or inhibition of metabolizing enzymes. Interactions are not necessarily consistent across route of exposure (Mumtaz and Durkin 1992). (Note that critical effects also may not be the same across routes.)

ATSDR's primary concern is exposure of human populations to contaminants from hazardous waste sites, which involves co-exposure to mixtures of contaminants from a medium such as groundwater for extended periods of time. There may be something of a sequential element as well if a population is exposed through more than one medium, but it is not well-approximated by the usual sequential exposure study in experimental animals, in which a single dose or short course of treatment with one chemical is administered prior to a single dose of a second chemical in order to determine the effect of the first chemical (often an enzyme inducer) on the toxicity of the second chemical. The "sequential" modifier, which lowers the numerical value of the weighting factor, is applied only when the data are considered less relevant to the expected human exposure. Because the sequence of exposure can have a substantial outcome on the nature of the observed interaction, it has been suggested that a BINWOE based primarily on toxicological observations from one sequence of exposure should, in general, not be applied to other sequences of exposure unless supported by data suggesting that such an application would be appropriate, even though the classification scheme contains a duration/sequence modifying factor (Durkin 1995). Thus, careful consideration of the applicability of the sequential data to simultaneous exposures is needed. The decision may be that the data are not applicable, or that they are applicable but should be given low confidence ratings, including the use of the sequence modifier.

In vitro assays generally are not used as the only source of information for the qualitative or quantitative assessment of interactions (Mumtaz and Durkin 1992). Rather, they are used as screening assays, and to provide information on potential mechanisms of interaction. If *in vitro* data are the only basis for documenting an interaction, it is probably most appropriate to consider this factor under the major rating of toxicological significance or mechanistic understanding. Depending on the quality of the *in vitro* assay, it may be appropriate to use a "b" modifier to further limit confidence in the assessment.

When the mechanistic and toxicological ratings do not fully convey the additional concerns for differences in duration, sequence, or route of exposure, it is appropriate to modify the confidence in the assessment by lowering the ratings (to 2, b, and ii) associated with these factors. In practice, when none of these ratings are lowered, they are simply omitted: for example, a BINWOE classification of <II.A.1.a.1 is expressed simply as <II.A.

Uncertainties. The BINWOE determination is an expression of confidence in a predicted direction of interaction. Sources of uncertainty are addressed by the main criteria (mechanistic understanding and toxicological significance) and by modifying factors within the alphanumeric BINWOE classification (differences in route, duration, and sequence of exposure). Any serious additional uncertainties that are not addressed in the different scoring elements should be mentioned in the text, along with a discussion of their potential impact on the assessment.

ISS Methodology

The ISS methodology (Woo et al. 1994) is somewhat analogous to the WOE methodology in that it uses data for binary mixtures to predict interactions in more complex mixtures. However, this method is designed for use only with carcinogens and is available as a dedicated software package with the appropriate data bases attached (containing approximately 1,000 carcinogens, promoters, and inhibitors in 60 structural and functional classes). The ISS calculates a weighting ratio reflecting the ratio of greater-than-additive to less-than-additive interactions for the components of a mixture. The estimation of the weighting ratio is based on the interactions data for the chemical pairs in the mixture and, for those pairs lacking interactions data, on interactions between other members of the chemical classes to which the chemicals belong. The weighting ratio also incorporates judgments as to the relative effectiveness of the four types of interactions (synergism, promotion, antagonism, and inhibition) in modifying the hazard. Weighting ratios greater than unity indicate that the combined effect of the mixture components is expected to be greater than additive, whereas ratios less than unity indicate that the combined effect is expected to be less than additive. Additional discussion of the ISS methodology can be found in ATSDR (2001). Because of the limitations of this method (e.g., too much weight on class-class interaction data), it is not recommended for assessing interactions, but can be used as a source of information on joint toxic action data for carcinogens (see the previous section on Locating Mixtures and Joint Toxic Action Data). ISS is undergoing further review and development, which may address the limitation regarding the class-class interactions, and update the database.

3. Recommendations for Exposure-Based Assessment of Joint Toxic Action of the Chemical Mixture

The purpose of this chapter is to present environmental health scientists with recommendations on how approach the exposure-based assessment of the potential hazard to public health, including how to incorporate concerns regarding potential additivity and interactions among the mixture components into the overall site assessment. The recommendations are based on the evaluation of the background information on the individual chemicals, presented in the Appendices, and the evaluation of mixtures and joint toxic action data performed in Chapter 2. The recommendations should be congruent with methods detailed in ATSDR (2001). Therefore, authors of interaction profiles must be thoroughly familiar with that guidance document and the methodologies described therein.

Figures 1 and 2 present overviews of the strategies for exposure-based assessment of joint toxic action of chemical mixtures on noncarcinogenic and carcinogenic effects. Detailed discussions of these strategies are provided in ATSDR (2001). The figures show how interaction profiles, and the various methods for incorporating concerns regarding additivity and interactions, fit into the overall scheme for mixtures assessment at a site. The interaction profile makes recommendations regarding how to approach assessment of a particular mixture, but the actual site assessment will be performed by an environmental health scientist or toxicologist using site-specific exposure data in concert with the approach recommended in the interaction profile and with ATSDR (2001).

As an example of the type of recommendation that may be made in an interaction profile, consider a mixture where the chemicals all affect the same target organ. Use of a hazard index approach may be appropriate, if the levels of exposure to two of more of the chemicals exceed certain criteria, as presented in Figure 1. To more adequately convey the potential health hazard associated with exposure to the mixture, the recommendation may be to apply the qualitative WOE methodology (ATSDR 2001). If the WOE method indicates that the joint action of most pairwise combinations of the chemicals is greater than additive, these data suggest that the mixture might have a more serious impact on human health than would be predicted based on the hazard index.

For another example, consider a mixture of three similarly acting chemicals where less-than-additive interactions have been demonstrated. The chemicals produce their effects by metabolism to reactive intermediates and simultaneous exposure to any two results in competitive inhibition of metabolism. A PBPK model is available that can predict the exposure levels necessary to produce competitive inhibition. In this case, the PBPK model can be used in conjunction with the WOE methodology for a more

sophisticated analysis. The recommendation to the environmental health scientist might be to compare exposure levels at the site with the apparent thresholds for interactions predicted by the PBPK model. At below-interaction-threshold levels, the joint action of the chemicals would be expected to be additive and a hazard index approach is appropriate. At higher levels, the joint action would be expected to be less than additive, so that a hazard index approach may overstate the health hazard, and would be conservative.

The recommendation for a mixture of three chemicals with different critical effects but with one or more sensitive effects in common would likely be to use the TTD modification of the hazard index approach,

Figure 1. Strategy for Exposure-Based Assessment of Joint Toxic Action of Chemical Mixtures: Noncarcinogenic Effects

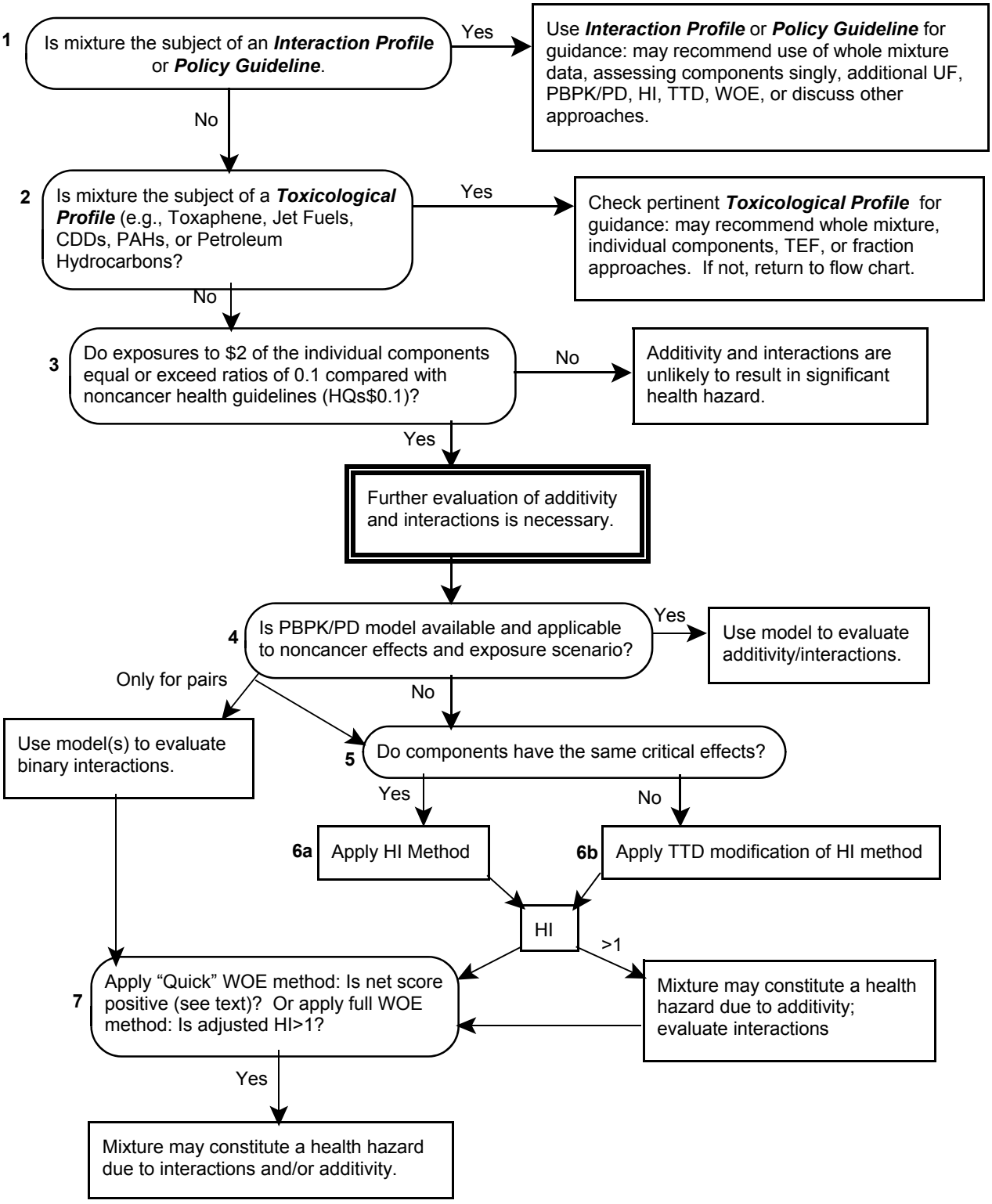
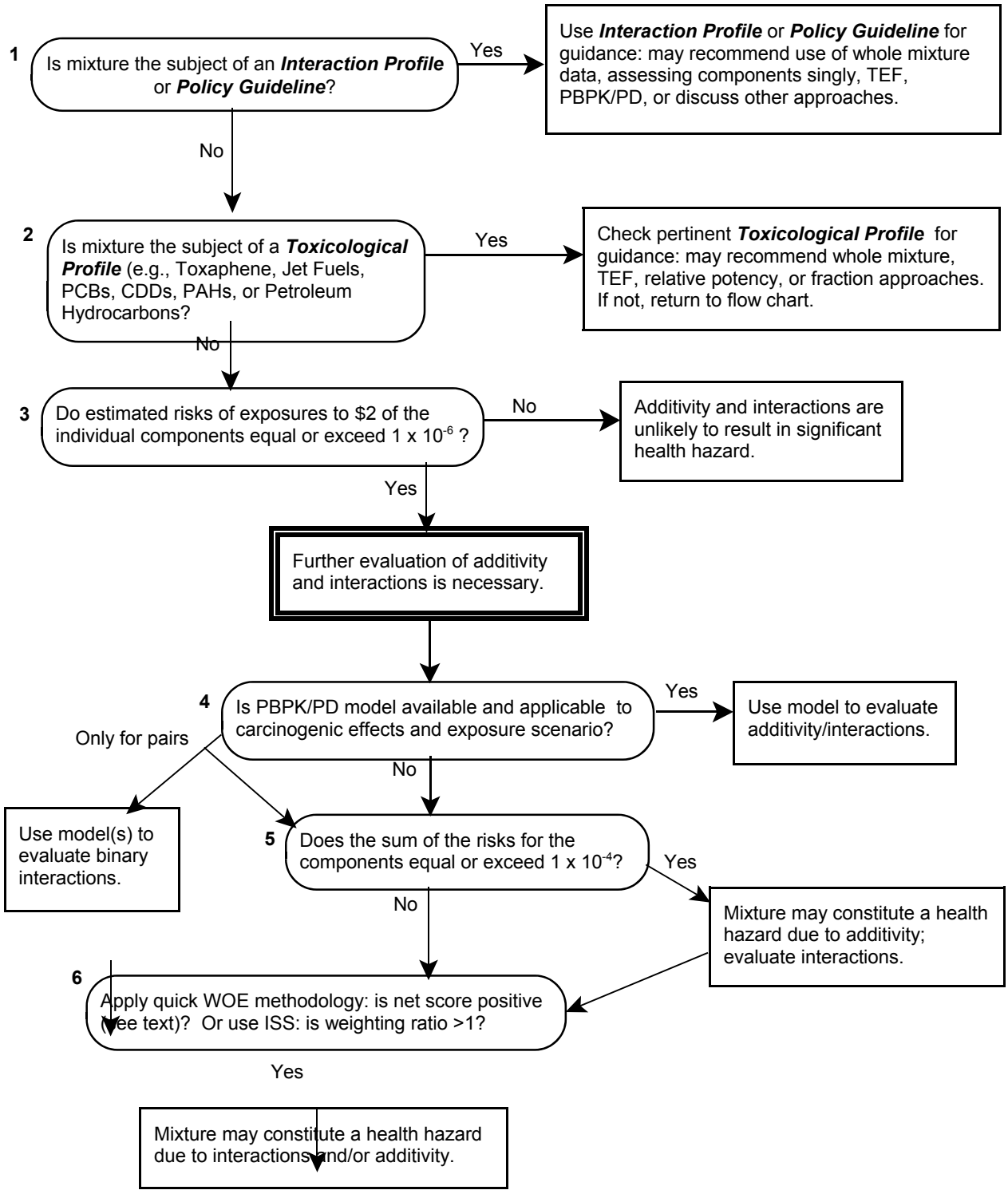


Figure 2. Strategy for Exposure-Based Assessment of Joint Toxic Action of Chemical Mixtures: Carcinogenic Effects



and to assess any unique critical effects with separate hazard quotients, depending on the exposure levels. The recommendation may also include application of the WOE method to take into account interactions, as per ATSDR (2001).

Recommendations for other situations might include using toxicity data to develop an MRL for the whole mixture, or summing the cancer risks and employing the qualitative WOE to predict hazard due to interactions. This is not meant to be a comprehensive list of possible outcomes, but merely to suggest some of the possibilities that would be consistent with the methodologies presented in ATSDR (2001). Keep in mind that the goal is to provide the environmental health scientist with a practical recommendation that can be readily implemented in the site assessment.

4. Conclusions

This section presents the conclusions of the interaction profile regarding the appropriate approach for exposure-based assessment of potential health hazards from exposure to the mixture of concern. It should be a page or less in length.

5. List of References

The intent of this section is to provide readers with a list of the literature regarding the joint toxic action of the mixture, so that those inclined to do so may follow up on the information presented. References for all literature cited in the profile should be marked with an asterisk in this section. Data regarding joint toxic action of the constituent chemicals of the mixture of concern generally should be cited to the primary reference. Inability to obtain or review a primary reference is not cause for citing the secondary reference. In such a case, the primary source should be referenced "as cited in" the secondary reference. Some exceptions may be advisable for less relevant data, such as *in vitro* data, or for mechanistic data, if voluminous. In these cases, a high quality review that provides an evaluation of the data may be cited. Background information on the toxicokinetics, health effects, and mechanisms of action of constituent chemicals (presented in the Appendices) should generally be cited to the relevant toxicological profile or other high quality secondary source. The format for references should follow that presented in the editorial guidelines contained in Appendix C to this guidance document. Copies of the references must be provided in alphabetical order to ATSDR.

Appendices: Background Information on Subject Chemicals

Purpose and Scope of Appendices

The appendices, one for each chemical component of the mixture of concern, provide the background information needed to interpret the joint toxic action data reported in the main body of the profile. Important topics of discussion for each appendix include toxicokinetics, health effects, mechanisms of action for the identified effects, existing health guidelines on both cancer and noncancer endpoints, and TTD derivations, if appropriate. A review of the most important issues relating to mixtures assessment for each of these topics is presented below. When writing the appendices, address all of the relevant issues for which data exist, but be concise. Each appendix should be no more than about 2–6 pages in length, unless TTD derivations are performed.

Include only well-documented information from reliable sources in the appendices. The primary source for background information on the constituent chemicals is the ATSDR toxicological profile series. The toxicological profiles present ATSDR's evaluation of the toxicokinetic and toxicity data for the individual chemicals, including derivation of MRLs and identification of mechanisms of action. If a toxicological profile is not available for a chemical, other secondary sources may be used. Look for sources that include all of the relevant topics and contain critical evaluations of the literature presented. In some cases, the toxicological profile or other review may present an issue in insufficient detail for the purposes of the interaction profile, particularly with regard to mechanism of action. In this case, it may be necessary to obtain the primary reference. When a primary reference is used, cite the primary reference and include it in the reference section. Otherwise, cite the information to the secondary source, or if it is useful to identify the primary reference, cite it and indicate in the reference list that the information was taken from a secondary source as in this example: (As cited in ATSDR 1997b).

Depending on how the mixtures of concern are chosen by ATSDR, there may be overlap in chemical constituents of these mixtures. Prior to starting work on the interaction profile, the contractor must find out from ATSDR if any of the chemicals in the mixture of concern have been included in previously completed interaction profiles. If so, the existing background information appendices on these chemicals should be obtained from ATSDR. Existing background information appendices can be used directly in the new interaction profile if the toxicological profile (or other source document) has not been updated. If the toxicological profile has been updated since the appendix was produced, then the appendix should be updated to reflect changes in the relevant sections of the updated source document.

The level of detail in the appendices should be sufficient to support evaluation of the interactions data presented in the main body of the text, particularly with regard to applicability of the interactions data to humans exposed at hazardous waste sites (presumably low level, repeated or chronic, simultaneous exposure). Study details that might typically be presented include species, exposure route, and general indications of duration (acute, intermediate, chronic) and dose (high, low). For oral studies, the method of administration (food, water, gavage, capsule) and vehicle may also be important. As a general rule, additional experimental details should be added only to the extent that they are needed to address specific issues with respect to the toxicokinetics, health effects, or mechanism of action of the chemical.

Toxicokinetics

The following guidance indicates information that may be included in this section, depending on the mixture components, the relevance to joint action, and the information available in the source document. Discuss the site(s) of uptake where the chemical enters the systemic circulation and, if known, percentages of the substance absorbed following oral or inhalation exposure. Identify any factors that are important determinants of absorption, such as vehicle for oral exposure, changes in the rate and amount of absorption over a range of doses, effects due to chemical form or method of exposure (e.g., in water versus food), and nutritional status of the dosed animals. Address the distribution of the chemical in major organs and tissues. Focus on localization and accumulation in target sites, particularly in repetitive dosing studies resulting in a steady-state. Mention if there are any differences in distribution or accumulation depending on route of exposure. Emphasize information on metabolic pathways that either convert the chemical to a form that is less toxic and/or can be readily excreted, or that produces a biologically active intermediate that is responsible for the toxic action (i.e., metabolic activation). Indicate detoxification pathways that are saturable as well as relevant species or strain differences in metabolism. An integrated discussion that can include *in vitro* data may be used if metabolism is not route-specific. Metabolic pathways can be described with a figure, *if needed* to clarify possible points of interaction for the mixture. In the absence of such figures, it may be useful to provide structures for organic components of the mixture, possibly as a separate appendix. Excretion data should be included for the principal excretory routes (urine, feces including bile, exhaled breath); other routes (e.g., hair and nails) can be discussed if pertinent to interactions toxicity. Indicate the half-life of the substance from target tissues and the body, as well as relevant inter- and intraspecies differences in excretion patterns. Note whether PBPK models have been developed to describe the disposition of the chemical in the body.

Health Effects

The main target tissues and health effects of each chemical should be discussed. Emphasize the most noted, sensitive, and/or significant effects of exposure in humans and animals, particularly those associated with repeated oral and inhalation exposures. Discuss targets of toxicity that overlap with those of other components of the mixture. Include information on developmental and reproductive toxicity and carcinogenicity as well as systemic effects, and note results of parenteral exposures if useful for supporting relevance to public health or in interpreting joint toxic action data. Indicate differences in effects between humans and animals and, if relevant, among animal species and strains. Note if there are delayed toxic effects and susceptible populations specific to the chemical. Mention any aspects of exposure that are important determinants of the likelihood for adverse effects (e.g., route, vehicle, duration, exposure level, or chemical form), as well as the potential impact of nutritional status, and whether the chemical is essential or beneficial to human health in some way.

Mechanisms of Action

Information on both pharmacokinetic mechanisms and mechanisms of toxicity are relevant to the discussion of mechanisms of action. Include pertinent data on mechanisms of absorption and distribution, such as processes by which the chemical crosses biological membranes (e.g., passive or active) and is translocated to target tissues (e.g., binding proteins such as metallothionein), as well as possible first-pass effects in the liver. Discuss if toxicity is associated with the parent chemical, its metabolite(s), or a combination of parent compound and metabolites, including the action of active or reactive metabolite(s). Include information on pathways of metabolism that become capacity-limited or saturated, particularly within the dose ranges used in the interaction studies, and formation of proximate or ultimate toxic metabolites in target tissues. Discuss what is known and hypothesized about the mechanism by which the chemical initiates organ toxicity, including information identified through structure-activity relationships. Mechanisms for both noncancer and cancer effects should be discussed. Discuss metabolic differences between species and the most appropriate animal model to use for extrapolation to humans. Consider possible implications of exposure duration on toxic interactions, such as possible adaptation to chemical exposure by induction of metabolic or DNA repair enzymes, and evidence to suggest that chronic exposure may lead to depletion of essential cosubstrates for metabolic elimination.

Health Guidelines

Inclusion of information on existing health guidelines is useful because it can help characterize the most relevant effects of concern for constituent chemicals. Chapter 7 or 8 of ATSDR toxicological profiles includes information on chemical regulations and advisories. This chapter summarizes ATSDR MRLs and the endpoints on which these levels are based, and tabulates (in Table 7-1 or 8-1) health guidelines and classifications for other agencies and organizations. Although information such as EPA toxicity (reference dose [RfD] and reference concentration [RfC]) values, EPA cancer slope factors, and EPA, National Toxicology Program (NTP), and International Agency for Research on Cancer (IARC) cancer classifications can be identified in the table, source references will have to be consulted for necessary information on their derivations. If a toxicological profile is not available for a chemical, then EPA, NTP, and IARC source references will have to be searched directly for relevant information.

Derivation of Target-Organ Toxicity Dose (TTD) Values

The derivation of TTDs for use in assessment of the joint toxic action of chemical mixtures is analogous to the derivation of MRLs, and should follow the applicable portions of ATSDR MRL guidance. TTDs are based on the other major characteristic effects of a chemical, which are known to occur at the same, or higher, exposure levels as the critical effects. Like the derivation of an MRL, the derivation of a TTD is not recommended for an endpoint that is affected only at the relatively high levels of exposure associated with severe effects. Because the purpose of TTD derivation is to support the estimation of endpoint-specific hazard indexes, TTD derivations should be performed for endpoints that are common to more than one component of a given mixture. In addition, endpoints identified as concerns in populations exposed to the mixture could be considered. Further detail and an illustration of the derivation and use of TTDs is provided in ATSDR (2001, Section 2.3.2).

In common with MRLs, TTDs are specific for route and exposure period. The TTD should be based on the highest no-observed-adverse-effect level (NOAEL) that does not exceed a lowest-observed-adverse-effect level (LOAEL) for the particular endpoint, as determined from the information in toxicological profiles, including the Levels of Significant Exposure Tables. If such a NOAEL is not available, the TTD would be based on the lowest LOAEL for that endpoint. Additional considerations, as for MRL derivation, are that the NOAEL or LOAEL used as the basis for the TTD should be from a representative, quality study, for the same route and exposure period as the TTD. When data for the exposure duration of concern are not available, a TTD derived for one duration may sometimes be applicable for other

duration(s) of the same route, if supported by the overall database. An additional uncertainty factor may be applied to extrapolate across exposure durations, based on scientific judgment. Dose adjustments and interspecies, intraspecies, and LOAEL to NOAEL extrapolation should be performed as for an MRL. When suitable data are available, and when appropriate, TTDs could also be derived using benchmark dose (BMD) modeling (Crump 1984, 1995; EPA 2001; Gaylor et al. 1998) to define the BMD, which is used in place of a NOAEL as the basis for TTD derivation, similar to the procedure for MRL derivation.

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EXHIBITS

Exhibit A. Title Page for Interaction Profile

DRAFT

INTERACTION PROFILE FOR [CHEMICAL A, CHEMICAL B, ..., CHEMICAL X]

Prepared by:

[Contractor Name]
Under Contract No. [ ]

Prepared for:

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

Month 20__

Exhibit B. Preface for Interaction Profile

PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found. The Food Quality Protection Act (FQPA) of 1996 requires that factors to be considered in establishing, modifying, or revoking tolerances for pesticide chemical residues shall include the available information concerning the cumulative effects of substances that have a common mechanism of toxicity, and combined exposure levels to the substance and other related substances. The FQPA requires that the Administrator of the Environmental Protection Agency (EPA) consult with the Secretary of the Department of Health and Human Services (which includes ATSDR) in implementing some of the provisions of the act.

To carry out these legislative mandates, ATSDR's Division of Toxicology (DT) has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists in collaboration with mixtures risk assessors and laboratory scientists have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

Exhibit C. Sample Summary Tables

Table x. Summary of Available Data on the Influence of Chemical A on the Toxicity/Carcinogenicity of Chemical B by Simultaneous Exposure						
		Results				
Duration	Endpoint	Greater than additive	Additive/No effect	Less than additive	Conclusions	References
Inhalation Exposure (ppm)						
Acute	Liver enzyme induction	10+0.5[a] (r) [b] 1+0.5 (m) 5+0.5 (m)			>additive	Smith et al. 1990 Kay et al. 1987 Jones et al. 1995
	Nasal lesions		150+25 (m)		additive	Parker et al. 1985
Chronic	Chem B levels in kidney		50+5 (r) 50+10 (r) 75+10 (r) 75+25 (r)		additive	Stevens et al. 1994 Chan et al. 1982 Jones et al. 1992 Baker et al. 1990
Oral Exposure (mg/kg/day)						
Acute	Chem B levels in kidney	250+10 (r)	150+10 (r) 150+10 (m)		additive at lower doses of A, >additive at higher doses of A	Jones et al. 1995 Smith et al. 1990 Kay et al. 1987

[a] dose for chemical A + dose for chemical B
[b] species code: r= rat, m= mouse

Table x. Summary of Available Data on the Influence of Chemical B on the Toxicity/Carcinogenicity of Chemical A by Simultaneous Exposure						
		Results				
Duration	Endpoint	Greater than additive	Additive/No effect	Less than additive	Conclusions	References
Inhalation Exposure (ppm)						
Acute	Liver enzyme induction	0.5+10[a] (r)[b] 0.5+1 (m) 0.5+5 (m)			>additive	Smith et al. 1990 Kay et al. 1987 Jones et al. 1995
Intermediate	Neuropathy		1+5 (r)		additive	Saylor et al. 1992

[a] dose for chemical B + dose for chemical A
[b] species code: r= rat, m= mouse

Exhibit D. Sample BINWOE Matrix

Table x. Matrix of BINWOE Determinations for [Type of Toxicity] from Simultaneous [Route] Exposure to Chemicals of Concern*

		ON TOXICITY OF			
		Chemical A	Chemical B	Chemical C	Chemical D
EFFECT OF	Chemical A		>IIIA2 (+0.25)	>IIB (+0.50)	>IIB (+0.50)
	Chemical B	>IIIA2 (+0.25)		=IIIC (0)	=IIIC (0)
	Chemical C	>IIB (+0.50)	=IIIC (0)		=IB2 (0)
	Chemical D	>IIB2 (+0.40)	>IIB2 (+0.40)	=1B2 (0)	

*Classification (numeric score)

Exhibit E. Format for BINWOE Determinations (adapted from Durkin, 1995)

Table x. Effect of **Chemical A** on **Chemical B**[: Type of Toxicity, e.g., Renal Toxicity]* for Simultaneous [Route]* Exposure

BINWOE: e.g., >II.B.2 (+0.40)*

Direction of Interaction - Briefly summarize your assessment of the direction of interaction (additive, greater than additive, less than additive, or indeterminate). This summary should include a concise statement of the specific criteria covered in the following sections. Any serious uncertainties should be mentioned. Applicability to critical effects and other sensitive effects, and to carcinogenic effects should be discussed as appropriate. Alternatively, separate endpoint-specific BINWOEs may be necessary.

Mechanistic Understanding - Briefly discuss why the particular rating for this scoring element was selected, or, if direction is categorized as indeterminate, discuss why mechanistic understanding does not support a prediction of direction of interaction.

Toxicological Significance - Briefly discuss the relevant data used as the basis for this scoring element and state why the rating was chosen, or, if direction is categorized as indeterminate, discuss why toxicological interaction data do not support a prediction of direction of interaction.

Modifying Factors - If any modifiers are used, discuss why these were chosen.

Additional Uncertainties - If you feel that the different scoring elements do not adequately address the uncertainties in the assessment, briefly discuss these uncertainties and how reasonable differences in judgment could affect the score.

*Specify route unless BINWOE is not route-specific. If needed, separate BINWOE ratings can be presented for different routes, durations, and types of toxicity, either in the same table or in separate tables, as considered appropriate. For example, if the same set of data is being used as the basis for conclusions regarding different types of toxicity, then a single table may be appropriate. If different sets of data are being used for conclusions for each type of toxicity, then separate tables are appropriate.

These BINWOE narratives should be focused so as to present the rationale for the BINWOE classification along with key references and key supporting information. The descriptions should be sufficient to support an understanding of the BINWOE determination.

Exhibit F: Table Summarizing BINWOE Classification Scheme

Table x. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

	Classification	Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	–1
?	Indeterminate	0
Quality of the Data		**Weighting**
	Mechanistic Understanding	
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
	Toxicological Significance	
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
	Modifiers	
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05

BINWOE = Direction Factor x Weighting Factor: Ranges from !1 through 0 to +1

*Source: ATSDR 2001a, 2001b

APPENDICES TO THE GUIDANCE DOCUMENT

Appendix A: Evaluating the Quality of a Toxicological Study

- Test material
  - Was it purchased or synthesized in-house?
  - Was the same lot used for all experiments?
  - Were any impurities present?
    If so, were the impurities removed?
  - Is the test material stable under experimental conditions?
    If not, were any adjustments made?
  - Was a vehicle used for administration?
  - Were the doses reported in the study?

- Animal selection
  - What is the rationale for the species selection?
  - Were the animals disease-free?
  - Is the animal model appropriate for the endpoint effects studied?
  - Optimal criteria at specific intervals:

	Acute	**Intermediate**	**Chronic**
Number of treatment groups	3–4	3	3
Number of animal groups	6–10	10–20	50/sex/treatment
Age of animals	>6 weeks	Young adult	Young adult
Control groups	Required	Required	Required

  - Are the species, strain, sex, age, treatment schedule, and vehicle the same for control as for treated animals?

- Study design
  - Are the route(s) expected for human exposures or other (inhalation, oral [diet, drinking water, gavage, other], dermal [intact, abraded, occluded])?
  - Is the exposure regimen daily, continuous, or intermittent (e.g., 6 hours/day, 5 day/week)?
  - Is the mortality loss for a chronic study no more than 5–10%?

- Optimal criteria at specific intervals:

	Acute	Intermediate	Chronic
Dose selection	At least 3	3	2 (MTD and LOAEL from a 90-day dose screen)
Period of exposure	Up to 14 days	15–364 days	365 days or more
Period of observation	Every 12–24 hours for 14 days	Every 12–24 hours	Every 24 hours
Body weight measured	Weekly	Weekly	Weekly up to 13; then every 2 weeks

- Endpoint effects
  - Were appropriate methods used to measure endpoint effects?
  - Are these methods state-of-the-art?
  - Was a dose-response relationship established?
  - Did the study sufficiently demonstrate a NOAEL or LOAEL?
  - Were appropriate statistical analyses performed?
  - Were the results statistically significant (at least $p<0.05$)?
  - Optimal criteria at specific intervals:

	Acute	Intermediate	Chronic
Organ weights recorded	Not specified	Liver, kidney, brain, gonads, heart, etc.	Liver, kidney, brain, adrenal, gonads, spleen, lung, etc.
Histopathological gross examination	Gross necropsy	Necropsy and histopathology for liver, kidney, heart, gross lesions, target organs	All tissue in at least control and highest dose group

Appendix B: Evaluating the Quality of an Epidemiological Study

I. Overall criteria

A. The study has been published or peer reviewed.

B. The paper should provide:

1. Background (i.e., supporting rationale, definition, and explanation of the problem).

2. Study objectives and study design, including assumptions, limitations, and statement of purpose or hypothesis.

3. Study population and control group (i.e., method of selection, rationale and criteria for inclusion/exclusion, appropriateness and limitations of control group).

4. Data collection method, including direction and possible magnitude of any bias introduced into the study (i.e., may be single-, double-, or triple-blind to prevent bias). QA, QC, or calibration data are presented for the data collection instrument (method).

5. Type and length of follow-up.

6. Account for (via matching, stratification, multivariate analysis, etc.) and clearly define major confounding factors.

7. Procedures and statistical methods used for data analysis. Significance levels need to display a strong association ($p<0.05$) to rule out the probability of the results occurring by chance variation.

8. Results that are related to the objectives of the study. Do the numbers in the tables add up?

9. Discussion of limitations and biases that may have affected the results. The examination of causality (conclusion) should be supported by the results.

10. A logical, temporal sequence of exposure-response that is toxicologically plausible.

11. A demonstrated dose-response relationship using valid estimates of exposure or dose.

II. Types of epidemiological studies

A. Observational studies

1. General points

a. These studies are rarely designed to provide quantitative risk information.

b. Groups are already divided on the basis of some experience or exposure (not created experimentally).

c. Sample size (N) should consider the size of the difference being detected (i.e., rare or common).

d. Small N does not mean study should not be done; rather, it might indicate that nothing could be found in the population. The study may need to state that numbers were too few to detect an excess risk.

2. Main types

a. Retrospective (case-control)

(1) These studies are helpful for monitoring substance/drug exposure.

(2) A positive association is demonstrated between the exposure and the disease/effect if the diseased group is more likely to be exposed than the group not diagnosed with the disease/effect. Researcher looks historically to determine exposure after the disease/effect has been determined.

(3) Cases:

(a) The study group must be delineated precisely, not generalized (e.g., premenopausal women and lobular breast cancer).

(b) Optimally, the study should use newly diagnosed cases with specified characteristics during a specified period in a defined population. Deceased cases as well as those alive when study is undertaken should be included.

(4) Controls:

(a) Controls should be representative of the general population in terms of probability and opportunity for exposure, and should represent the population from which cases arose.

(b) Individual matching is optimal.

(5) Advantages:

(a) The number of subjects can be small because the study is initiated by the identification of cases.

(b) More than one risk factor in the same set of data can be identified.

(c) Studies can take into consideration changes in exposure.

(6) Disadvantages:

(a) Information on past events may be inaccurately recorded or not available.

(b) Information supplied by an informant may be consciously or unconsciously biased.

(c) The study yields only an odds ratio that is an estimate of relative risk (i.e., a comparison of incidence for exposed versus unexposed populations). It is advisable to select more than one control group.

b. Prospective (cohort or longitudinal)

(1) Cohort is free of disease/effect but varies in exposure to the supposed factor. The exposed group is then followed to see if the disease/effect develops. The assumption is that exposed individuals are representative of all exposed persons regarding the risk of disease/effect development.

(2) A positive association is demonstrated between the exposure and the disease/effect if the exposed group develops the disease/effect at a greater rate than those not exposed.

(3) Cohort needs to be as similar as possible to the group it is intended to represent.

(4) Advantages:

(a) Permits calculation of incidence rates among exposed and not exposed. Incidence = number of new cases/total population at risk.

(b) Permits observation of many outcomes.

(5) Disadvantages:

(a) Long-term follow-up may be difficult.

(b) Large cohort (study group) is expensive.

c. Historical prospective

(1) Combines advantages of retrospective and prospective.

(2) Follows historically identified healthy exposed and unexposed cohorts for the development of disease/effect.

(3) Can calculate actual incidence and relative risk.

d. Cross sectional (prevalence): Both risk factors and disease are determined at the same time (e.g., prevalence of CHD and serum cholesterol level).

B. Experimental studies: General points

1. The impact of varying some controlled factor is studied.

2. These studies are not common, for obvious reasons.

3. Subjects should be divided into treatment groups by random allocation.

C. Occupational studies

1. Ecological

a. Generate hypotheses.

b. A group rather than individual is the unit of comparison.

2. Cross sectional (prevalence)

    a. Observations of a group are made at one point in time, yielding prevalence rates. Prevalence = number of old and new cases/total population at risk.

    b. These studies represent one of the most frequently used ways of identifying a disease/effect in a community (survey, screening).

    c. Health effects of short duration are less likely to be found than cases of long duration.

    d. These studies are especially suited for subtle, subclinical health effects for which records are unlikely to exist.

    e. The relationship between effects and time cannot readily be explored.

3. Case control

    a. These studies are used when the disease/effect of interest is relatively rare and would require a large cohort for follow-up.

    b. Environmental concentrations and biological levels are often measured.

    c. Several occupations or substances may be associated with the disease/effect of interest.

    d. The influence of various modifiers can be studied (synergism).

    e. Previous jobs are often of greater relevance than current, therefore entire work history needs examination.

4. Cohort

    a. Occupational cohort studies are usually mortality studies.

    b. Cohort should be defined as broadly as possible, prevalence or incidence.

    c. Eliminating workers from the cohort who are not active can lead to serious biases in assessing mortality because this can distort the age distribution of the cohort and omit workers who left because of ill health.

d. Dose-response relationships or high-risk jobs are searched for by dividing cohort into exposure level groups.

Appendix C. Editorial Guidelines

General Formatting

1. Document typeface should be Times New Roman, 11 pt with 1.5 line spacing.

2. The body of the text should be 1 inch from the edges of the paper (top, bottom, left, and right edges). Page headers, footers, and numbers should fall within this 1 inch.

3. Left justification (not full) should be used.

4. Page numbering should be set to the "top right" position.

5. A table of contents should be created for each draft. Some of the lines may appear too long. At least two dots must appear at the end of each line. This should be corrected for finals but need not be corrected for drafts.

6. In the list of tables and list of figures, the table and figure numbers and titles should be listed for all drafts. However, the page numbers need not be included until the final.

7. The following footer should appear on the left side of all pages of drafts.

*** DRAFT — DO NOT CITE OR QUOTE — Month ___, year ***

No footer should appear in the final version of the document.

Capitalization

1. Use lower case for the following words and expressions:

federal (except when part of a proper name, e.g., Federal Communications Commission)
Henry's law constant
lowest-observed-adverse-effect level
no-observed-adverse-effect level
state (except when part of a proper name, e.g., Indiana State Water Control Board)

2. Use upper case for proper names and for titles.

Italics

1. Use italics rather than underlining. (Exception: Because of a font or printer problem, italics may not be possible; in these rare instances, continuous underlining may be used.)

2. Italicize technical phrases in a foreign language, e.g., *in vivo, in vitro, in utero, ad libitum, in situ.* Italics need not be used for foreign phrases used by the general public, e.g., a priori, rigor mortis, et al.

Abbreviation

1. Be consistent in the use of acronyms, abbreviations, and symbols.

2. Don't abbreviate common terms such as central nervous system, subcutaneous, intraperitoneal, and gastrointestinal.

3. When an abbreviation or acronym is to be used in a profile, the word or phrase should be spelled out the first time it is used, followed by the abbreviation or acronym in parentheses.

   However, the abbreviation U.S., when used as an adjective, need not be spelled out the first time it is used. United States as a noun should always be spelled out.

4. Most units of measure should be abbreviated. However, in text, it is preferable *not* to abbreviate *common* units of measure such as second, minute, hour, day, week, month, year, pound, ounce. In addition, in text, the unit "metric ton(s)" should normally be written out rather than abbreviated as kkg. Use L for liter.

5. Substance names should be spelled out in the text of the profiles, not abbreviated. Exceptions to this can be made on a case-by-case basis and depend on the prevalence of an "accepted" abbreviation. If you use an abbreviation for a substance, spell it out the first time and insert the abbreviation in parentheses after it.

6. Many analytical methods may be abbreviated using accepted abbreviations but should be spelled out the first time, e.g., gas chromatography (GC), mass spectrometry (MS), high-performance liquid chromatography (HPLC).

7. On first occurrence, spell out a species name. Thereafter, the species name should be abbreviated.

First occurrence	**Subsequent occurrences**
Escherichia coli	*E. coli*
Salmonella typhimurium	*S. typhimurium*

8. Do not use postal abbreviations or any other abbreviations for states, except in tables, where postal abbreviations are acceptable.

Hyphens, Em Dashes, En Dashes, and Symbols

1. There are three types of hyphens:

hyphen (-)	Always visible; allows the characters after it to go to the next line when necessary (e.g., treatment-related)
soft hyphen (Ctrl, -)	Invisible when not needed; appears and forces characters after it to go to the next line when necessary (long words such as "nitrosodiphenylamine" use a soft hyphen)
hard hyphen (Home, -)	Always visible; forces the characters before and after it to remain together on the same line (e.g., X-ray); a hard hyphen should always be used with exponents (e.g., 10^{-4})

An example in which all three would be used:

N-nitrosodiphenylamine-induced effect
8 8 8
hard hyphen soft hyphen hyphen

2. Use a hyphen between two words that work together to modify a noun:

dose-response relationship time-weighted average
acute-duration exposure DDT-contaminated fruit

Exception: Do not use a hyphen with "sister chromatid," as in "sister chromatid exchange."

3. Use an em dash (Control W, Key = 4,34) with no spaces on either side to set off part of a sentence.

> The information in this section is organized first by route of exposure—inhalation, oral, and dermal—and then by health effect—death, systemic, immunological, neurological, etc.

4. Use an en dash (Control W, Key = 4,33) when expressing a range of numbers (e.g., 16–24); this is not required in the bibliography or any other section of the document for which data is generated by another program. An en dash is the width of the letter n; an em dash is the width of the letter m. With certain fonts, printer selections, or printers, symbols such as the em dash and the en dash may be incorrectly produced. Word processors, proofreaders, editors, and authors should be aware of the difference in appearance and use and see that corrections are made when necessary.

5. In a range of numbers, use the word "to" only after the word "from." (If you use "between," then you must use "and.")

   16–24-day-old mice
   the rats were administered 16–24 mg/kg/day
   the mice were administered doses ranging from 16 to 24 mg/kg/day
   between day 10 and day 13

6. In most cases, use symbols for alpha, beta, delta, gamma, and other Greek letters or mathematical terms. (Exception: When the word normally is written out, e.g., gamma-globulin, spelling out is acceptable.)

7. Don't surround mathematical symbols with spaces (e.g., $p<0.01$, $1x10^{-6}$).

8. Use round, not square, bullets (Control W, Key = 6,34).

9. To produce the Greek mu symbol (μ), use Control W, Key = 8,25.

Line Endings

1. Hyphenation normally should not be used in the profiles. However, a soft hyphen (see "Hyphens," item 1) can be used in a word at the end of a line to help improve a line on which the words appear too close together or too far apart.

2. If hyphenation is used, compound substance names should be divided after a complete element.

tetrachloro-	or	tetrachlorodibenzo-
dibenzo-*p*-dioxin		*p*-dioxin

   A substance name that starts with one letter or number followed by a hyphen (e.g., 2-hexanone) should *not* be divided after that hyphen.

3. Two or more words separated by a slash can be divided over two lines by inserting a soft return (Home, Enter) after the slash, so the slash remains at the end of the first line.

   . . . . . quality assurance/
   quality control

4. Use a hard space (Home, space) between numbers and units of measure and an en dash (Control W, Key = 4,33) between ranges of numbers to avoid splitting these terms at the end of a line.

5. Do not split the day and month over two lines. Use a hard space (Home, space).

Don't

. . . . . March
6, 1989 . . . . . .

Do

. . . . . March 6,
1989 . . . . . .

6. Do not split numbers and units of measure over two lines. Use a hard space (Home, space).

Don't

. 24
mg of substance x . . . . . .

Do

. . . . . 24 mg
of substance x . . . . . .

7. Use a hard space to keep the words "Chapter," "Figure," "Table," and "Section" on the same line with the numbers that follow them.

Don't

. . . . . Section
2.3.1 . . . . . .

Do

. . . . . .Section 2.3.1
. . . . . .

8. Use a hard hyphen (Home, -) to keep table and figure numbers together

Don't

. . . . . Table 2-
1. . . . . .

Do

. . . . . Table 2-1

Numbers

1. Use a comma in a four-digit number in text. In tables where space is limited, the comma is not required. Be consistent within a table.

2. Units of measure should be consistent throughout the profile (to the extent possible). For example, do not say "x mg/m^3 and y ppm." The mg/m^3 should be converted to ppm or vice versa.

3. Do not spell out the numbers 10 and higher, except when the number is the first word in a sentence.

   The study included 12 rats and 45 mice.
   24 mg/kg/day
   Twelve mice died before the end of the . . . .

   Spell out numbers less than 10 except when they are used with a unit of measure or when they are in the same sentence with a number of 10 or more.

   The study included one mouse and two rats.
   The rats were observed for 1 week.
   The study included 12 mice and 1 rat.

   Numbers in the millions and billions are expressed using the words "million" or "billion" preceded by the appropriate number, either in numeral form or spelled out, whichever is appropriate according to the above guidelines.

4. Use scientific notation to represent numbers with more than three zeros after the decimal point. Use either a multiplication symbol (Control V, Key = 6,39) or a lower case x, but be consistent in the use of one or the other throughout each profile. Do not put spaces before and after the multiplication symbol or x. Do not use the "E" method of abbreviating these numbers.

   1×10^{-6} (this is the multiplication symbol)
   $1x10^{-6}$ (this is the lower case x)

5. When a symbol or unit is used with a range of numbers, use the symbol or unit only after the second number in the range.

   40–60%
   10–15 EC (use a hard space between 15 and E)
   10–20 mg.

6. In a series of numbers with symbols or units, use the symbol or unit only after the last number.

    40, 50, and 75%
    10, 140, or 500 mg/kg

Tables and Figures

1. Put tables and figures in numerical order according to their order of citation in the text.

2. Tables and figures do not need to be inserted into the text for drafts, only for the final or camera-ready version of the document.

3. The summary table(s) for each binary mixture in Section 2.2 are placed at the end of the section of text that deals with that mixture.

4. The BINWOE determination tables are placed at the end of Section 2.3. The table summarizing the BINWOE classification scheme (Exhibit F) should immediately precede the BINWOE determinations.

5. Text entries in a column should be flush left. Tables should be created with the Table feature of WordPerfect, not with tabs. All numerical entries should be lined up along the decimal point (when text and numbers are mixed, use flush left).

Footnotes and Definitions in Tables and Figures

1. There should be one line between the line at the end of the table and the footnotes. Footnotes must be lettered in the order in which they appear in the table or figure, going from top to bottom and from left to right across the columns. Footnotes and definitions should appear on the last page of tables that continue for 2 or more pages.

2. In tables, footnotes are lettered; in figures, footnotes use asterisks.

3. In tables and figures, put a period after footnotes that are sentences. Footnotes consisting of short phrases that are not sentences should not have a period. Definitions at the end of tables or figures never end with a period.

4. Definitions should be placed on the last page of the table after the footnotes (beneath the line at the end of the table). They should be listed in alphabetical order and separated by semicolons.

    DAN = 2,3-diaminaphthalene; ip = intraperitoneal

Table and Figure Titles

1. Use boldface print for table and figure titles except when shown otherwise in the exhibits.

2. In the case of a wrap-around title, center both lines individually, with the second line shorter than the first line. The following is the correct format for table and figure titles:

Table x. Summary of Available Data on the Influence of Chemical A on the Toxicity/Carcinogenicity of Chemical B by Simultaneous Exposure

3. In table and figure titles, capitalize the first and last words and all nouns, pronouns, adjectives, verbs, adverbs, and subordinate conjunctions. Do not capitalize articles (the, a, an), coordinate conjunctions (and, or), prepositions (regardless of length), and the "to" in infinitives.

4. The title at the top of all continuation pages of tables and figures should appear as shown below.

Table x. Summary of Available Data on the Influence of Chemical A on the Toxicity/Carcinogenicity of Chemical B by Simultaneous Exposure (*continued*)

5. The table title should be the first row of the table.

Text Citations

1. Basic citation: (Smith 1978).

2. Two authors: (Light and Wong 1981).

3. Three or more authors: (Meredith et al. 1979).

4. If a reference carries no author on the title page but is published or sponsored by a government agency, corporation, association, or other group.

   (EPA 1988) or (ACGIH 1986) or (Dow 1983)

   Names such as Dow Chemical Co. or Velsicol Chemical Co. should be abbreviated in the citation in the text (Dow or Velsicol).

5. For drafts, when citing a study that is not yet in-house, cite the study as you would any other study. (The words "As cited in . . ." or "Retrieval in progress" will appear in the bibliography—see "Bibliography," item 7.)

6. Separate citations with semicolons, not commas, in text and tables.

7. If there is a letter after the year in the bibliography, include it in the citation.

   (EPA 1980a, 1980b) or (Smith et al. 1982a, 1984a, 1984b)

8. Two or more citations within parentheses in the text should appear in alphabetical order.

   (Brown 1988; Light and Wong 1981; Smith 1978, 1988)

9. References by the same author(s) should be ordered chronologically within parentheses.

   (Suter 1973, 1975b, 1988)

10. When there is more than one article by the same author in one year, do not repeat the author's name.

    (Suter 1975a, 1975b)

11. When the author's name is part of the sentence:

    A study by Smith and Jones (1980) showed that . . .

    Smith et al. (1989) reported an increase....

12. When citing an anonymous report, write out the word "anonymous."

    (Anonymous 1981)

13. Personal written communications are cited in the text by name and year (see also the "Personal Written Communications" subsection of "Bibliography," below).

    (Silverman 1983)

14. The citation must appear immediately after the material cited. If one sentence has two citations referring to different elements in the sentence, you must be specific.

    . . . was found to be hepatotoxic in rats (NTP 1980) and in mice (NTP 1987).

15. Databases should be cited by the name or acronym of the database as it appears in the reference section. See Bibliography section #3.

    (HSDB 1988)

Reference List

1. Information in references is usually (but not always) in the following order.

    Author
    Year
    Title
    Edition number
    Publisher name/location or location/name (see examples)
    Volume, issue (see examples)
    Page(s)

    Follow the examples that appear throughout this section when assembling a reference list.

2. If page numbers are included at the end of a reference, do not use the word "page(s)" or its abbreviation.

3. If an acronym is used as the author, spell out the words in the reference.

    HSDB. 1988. Hazardous Substances Data Bank. National Library of Medicine, National Toxicology Information Program, Bethesda, MD. July 18, 1988.

    ACGIH. 1993. 1993-1994 Threshold Limit Values for chemical substances and physical agents and biological exposure indices. American Conference of Governmental Industrial Hygienists. Cincinnati, OH.

    In rare cases, what appears to be an acronym is not.

    HazDat. 1997. Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA. February 13, 1997.

4. Transcribe titles exactly. Keep punctuation and unusual spellings as they appear in the original. If a word in a title is italicized or underlined, italicize it.

5. Designate unpublished studies requiring peer review by inserting the following at the end of the reference.

    [unpublished study to be peer reviewed]

    If the study is accepted for inclusion in the profile after peer review, remove the above statement. If unacceptable for inclusion, delete the entire citation.

6. For drafts, when citing a study that is not yet in-house, at the end of the reference insert the words (Retrieval in progress).

   However, if an article to which you are referring was mentioned in another article in the reference list, use the words (As cited in . . .) instead—it is assumed that retrieval is in progress.

   Get the article and insert the complete information in the reference list by the second draft, if possible.

7. Do not split a reference over two pages.

8. When more than one study would have the same citation in the text, letter the references (a, b, c, d, etc.) according to the instructions in "Alphabetization," below.

   Smith AB, Harris CD, Reed FG, et al. 1980a. . .
   Smith AB, Jones FD, Apolo GH. 1980b. . .

9. If a study has more than three authors, list only three and add the words "et al."

   Smith AB, Jones FD, Apolo GH, et al. 1980a. . .

Alphabetization

1. References should be alphabetized according to the first word, which will be either the first author's last name or the first word of the name of the organization.

2. When the first author's last name (or the organization name) is the same for more than one study, use the following guidelines.

   Put all the single-author studies first, then the double-author studies, then the studies with three or more authors. Within these three categories, use the following alphabetization priorities.

   Single-author studies

   1. Author's last name
   2. Author's initials
   3. Year (oldest study first)
   4. Article title

Example:

Sampson AB. 1985. . .
Smith AB. 1980a. Dibromoethane . . .
Smith FG. 1975. . .
Smith FG. 1980b. Benzene . . .
Smith FG. 1980c. Chloroethane . . .

Double-author studies

1. First author's last name
2. First author's initials
3. Second author's last name
4. Second author's initials
5. Year (oldest study first)
6. Article title

Example:

Sampson AB, Brown MN. 1985. . .
Smith AB, Jones KL. 1960. . .
Smith AB, Jones KL. 1965. . .
Smith AB, Klein EF. 1975a
Smith AB, Klein GH. 1975b
Smith CD, Apolo HJ. 1980. . .
Smith CD, Klein EF. 1975c. Dibromoethane . . .
Smith CD, Klein GH. 1975d. Benzene . . .
Smith CD, Klein GH. 1975e. Chloroethane . . .

Studies with three or more authors

1. First author's last name
2. First author's initials
3. Second author's last name
4. Second author's initials
5. Third author's last name
6. Third author's initials
7. Year (oldest study first)
8. Article title

Example:

Sampson AB, Brown MN, Lopez OL. 1975. . .
Smith AB, Apolo HJ, Klein EF. 1970a. . .
Smith CD, Apolo HJ, Klein EF. 1970b. . .
Smith CD, Klein EF, Jones EF. 1970c. . .
Smith CD, Klein GH, Jones EF, et al. 1970d. . .
Smith CD, Klein GH, Lloyd FG. 1970e. Dibromoethane . . .
Smith CD, Klein GH, Lloyd HI. 1970f. Benzene . . .
Smith CD, Klein GH, Lloyd HI. 1970g. Chloroethane . . .
Smith FG, Jones KL, Klein EF, et al. 1960. . .

3. Once alphabetization has been accomplished and letters have been added after the year when necessary, if a new reference must be added and requires a letter, the new reference should be added at the end of the list instead of in its proper alphabetical place. This way it will not be necessary to reletter the references.

Capitalization and Punctuation

1. Capitalize the first letter of all words in the title of a journal.

2. Capitalize the first letter only of the title of a book, chapter in a book, report (government or otherwise), or article in a journal. (Exception: proper names, e.g., "The Merck index.")

3. If there is a subtitle and no punctuation in the original after the primary title, use a colon, skip two spaces, and capitalize the first letter of the first word of the subtitle. If the original title has an em dash or hyphen, leave it in and do not capitalize the following word.

   Smith WP, Jones HW, Harris CP. 1930. Acute response of guinea pigs to vapors of various compounds: Dioxin and other new commercial organic compounds. Public Health Rep 99:23-32.

   Stahl CJ, Fatteb AV, Dominguez AM. 1969. Trichloroethane poisoning—observations on pathology and toxicology in six fatal cases. J Forensic Sci 14:393-399.

4. Do not use a period with journal abbreviations or authors' initials.

5. Do not use commas in front of authors' initials.

Journal Articles

1. Journal titles should always be abbreviated according to the standard abbreviations for journal titles in *Index Medicus*. If the journal title is not in *Index Medicus,* use the *List of Serials Indexed for Online Users—1989* (National Library of Medicine). The abbreviation must be copied exactly. If a journal does not appear in either of these two lists, use the whole name of the journal. Do not make up abbreviations.

   Kawaji M, Pai HL, Phillips CR. 1981. Use of gross filter activities in a continuous working level monitor. Health Phys 40:543-548.

2. Articles in journal supplements should be referenced as follows.

   Mastri ARE. 1980. Neuropathy of diabetic neurogenic bladder. Ann Intern Med 92(Pt 2):316-318.

3. Use the word "Anonymous" when no author is given.

   Anonymous. 1981. Coffee drinking and cancer of the pancreas [Editorial]. Br Med J 283:628.

4. If a journal article is unpublished, state whether the article is "submitted" for publication or "accepted" for publication; do not use "in press" because of the term's ambiguity. If a "submitted" article has not been accepted, it should be removed from the list before release of the profile because it has not been peer reviewed.

   Young JD, Chenoweth MD, Braun WH. 1988. The pharmacokinetics of 1,4-dioxane in humans. [Accepted for publication by Toxicol Appl Pharmacol].

Abstracts

Current abstracts should be discussed in the "Ongoing Studies" sections of Chapters 2, 5, and 6. In almost all cases, abstracts should be disregarded if not followed up in the literature. Citation of an abstract in a profile requires the ATSDR chemical manager's approval. (Such approval will be based primarily on its significance in the profile and the lack of primary literature.) Abstracts should be referenced as follows.

Kerby GP. 1955. Occurrence of acid mucopolysaccharides in human leukocytes [Abstract]. J Clin Invest 34:944.

Translations

1. Foreign language articles should be sent to ATSDR for translation.

2. Until the article is translated, insert the following at the end of the reference: [Translation in progress]. Remove this once the translated article has been received and reviewed.

3. Enclose the translated title in brackets; indicate the original language in parentheses at the end of the reference.

    Wurtz A. 1982. [New investigation on ethylene oxide.] Ann Chem Pharm 122:354-359. (German)

Books

1. A book with an author is referenced as follows.

    Shapiro J. 1981. Radiation protection—a guide for scientists and physicians. 2nd ed. Cambridge, MA: Harvard University Press, 50-61.

2. A book with an editor is referenced as follows.

    Stolman A, ed. 1969. Progress in chemical toxicology. New York, NY: Academic Press, 42-49.

    If a book has more than one editor, list each editor, up to three. If there are more than three editors, use "et al." after the third editor's name. Use "eds." rather than "ed." to indicate multiple editors.

    Ourisman A, Katz RF, Holmes DR, et al., eds. 1969. Progress in chemical toxicology. New York, NY: Academic Press, 93-74.

3. A chapter in a book must be cited by the author of the chapter if there is one. If not, the chapter must be cited by the author or editor of the book.

    Chameaud J, Perraud R, Masse R, et al. 1981. Contribution of animal experimentation to the interpretation of human epidemiological data. In: Gomez M, ed. International Conference: Radiation hazards in mining. New York, NY: Society of Mining Engineers of American Institute of Mining, Metallurgical, and Petroleum Engineers, Inc., 222-227.

Personal Written Communications

Use references to personal written communications only when necessary. A copy of the communication must be provided (see also "Text Citations," item 14).

Silverman MF. 1983. Written communication (September 30) to Wynn Oliver, Tishman West Building Management Corporation, regarding cleanup levels following a PCB transformer fire. Department of Public Health, City and County of San Francisco, CA.

Corporate and Organizational Reports

Corporate reports and organizational reports should be referenced as follows.

Fiedlander BR, Pifer JW, Hearne FT. 1985. 1964 Methylene chloride cohort mortality study: Update through 1984. Eastman Kodak Company, Health and Environment Laboratories, Epidemiology Section, Rochester, NY. NTIS publication no. 124332434.

ACGIH. 1993. 1993-1994 Threshold Limit Values for chemical substances and physical agents and biological exposure indices. American Conference of Governmental Industrial Hygienists. Cincinnati, OH.

AIHA. 1966. Cobalt: Except the carbonyls. In: Hygienic guide series. American Industrial Hygiene Association. Akron, OH.

Provide NTIS numbers, EPA OTS numbers, or other useful locator codes where possible.

Government Documents

Government documents and publications should be cited by the agency, even if prepared by contractor authors.

CCEHRP. 1985. Risk assessment and risk management of toxic substances. Report to the Secretary, Department of Health and Human Services (DHHS), Washington, DC, by DHHS Committee to Coordinate Environmental Health and Related Programs.

EPA. 1979. U.S. Environmental Protection Agency: Part III. Federal Register 44:15874-15920.

IARC. 1980. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Vol. 22: Some sweetening agents. World Health Organization, Lyon, France.

NTP. 1984. National Toxicology Program—technical report series no. 248. Toxicology and carcinogenesis studies of 1,3-butadiene (CAS No. 106-99-0) in B6C3F_1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH publication no. 84-2544.

NIOSH. 1983a. Criteria for a recommended standard—Occupational exposure to styrene. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. DHHS (NIOSH) publication no. 83-119.

NIOSH. 1983b. Health hazard evaluation—determination report no. 83-395. Overnight Transportation Company, St. Louis, MO. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health.

OSHA. 1982. U.S. Department of Labor. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.134, OSHA 2206.

Provide NTIS numbers, EPA OTS numbers, or other useful locator codes where possible.

Databases

1. References to databases are as follows.

   HSDB. 1997. Hazardous Substances Data Bank. National Library of Medicine, National Toxicology Information Program, Bethesda, MD. July 18, 1997.

   HazDat. 1997. Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA. February 13, 1997.

2. Provide a printout of the information on the day the database was accessed.

Conference Proceedings

Conference proceedings should be referenced as follows.

Smith J. 1987. Soil and groundwater contamination at wood preserving plants. In: Bell DR, ed. Proceedings of the 41st Industrial Waste Conference, Purdue University, May 13-15, 1986. Chelsea, MI: Lewis Publishers, Inc., 347-351.

Miscellaneous

1. Words with prefixes should be closed up rather than hyphenated (e.g., nonvolatile, nonresponsive, nonneoplastic, multiphase, pretreatment, postexposure, undiluted). If the absence of a hyphen would create confusion regarding the meaning of the word, use the hyphen, e.g., un-ionized (not unionized).

2. Use database, endpoint, waste water, and groundwater.

3. Chapters should be referred to in the text as chapters, not sections. Sections within a chapter should be referred to as sections.

    . . . is discussed in Section 2.2.3.
    . . . is discussed in Chapter 2.

    When referring to a section or chapter, do not include the name of the section or chapter unless it does not have a number. For example:

    . . . the discussion of Respiratory Effects in Section 2.2.1.2.

4. When a person's name is cited in the text, use periods and no space with his/her initials: E.R. Brown.

5. Avoid using "man" for "human(s)."
   Avoid "human volunteer" (omit the word "human")—animals rarely volunteer.

6. Avoid numbering or lettering items within text:

    Don't

    The routes of exposure discussed in this section are (1) oral, (2) inhalation, and (3) dermal.

    Do

    The routes of exposure discussed in this section are oral, inhalation, and dermal.

    If you do number or letter items in text, always use parentheses in pairs.

    (1) and (2) not 1) and 2)
    (a) and (b) not a) and b)

7. Use a comma after each member within a series of three or more words, phrases, letters, or figures used with "and," "or," or "nor" (e.g., "red, white, and blue").

8. Use brackets within parentheses, not parentheses within brackets, unless you can recast the sentence to avoid the situation.

9. Avoid using a slash (/) between spelled-out words. Use one of the following as appropriate: per, and, or, and/or. A slash is usually appropriate, however, with units of measure, e.g., mg/kg/day. If you do use a slash between spelled out words, put an invisible soft return (ISR) (Home, Enter) after it when appropriate (e.g., use ISR in physical/chemical, don't use ISR in mg/kg/day).

10. Use "that" to introduce restrictive clauses. A restrictive clause is a clause that is essential to define the word or phrase it modifies. (Hint: If the sentence would be incomplete or if the intended meaning of the sentence would not be expressed without the clause, use "that.") Example: The book that you ordered is out of print.

11. Use "which" to introduce nonrestrictive clauses. A nonrestrictive clause is a descriptive clause that is not essential to define the word or phrase it modifies. (Hint: If you can put a comma before the clause, you should use "which.") Example: Her house, which is in Sarasota, is beautiful.